Eesti Maaülikool
Estonian University of Life Sciences

# CHANGES IN THE BIOCHEMICAL COMPOSITION OF APPLE (*Malus domestica* Borkh.) FRUITS DEPENDING ON ROOTSTOCK AND CALCIUM TREATMENT

## MUUTUSED ÕUNTE BIOKEEMILISES KOOSTISES SÕLTUVALT AED-ÕUNAPUU (*Malus domestica* Borkh.) POOKEALUSEST JA KALTSIUMIGA VÄETAMISEST

**LEILA MAINLA**

A Thesis
for applying for the degree of Doctor of Philosophy
in Agriculture

Väitekiri
filosoofiadoktori kraadi taotlemiseks põllumajanduse erialal

Tartu 2013

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# LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following research papers, which are referred to by their Roman numerals:

I Moor, U., Karp, K., Põldma, P., Asafova, L., Starast, M. 2006. Post-harvest disorders and mineral composition of apple fruits as affected by pre-harvest calcium treatments. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science* 56 (3): 179–185.

II Mainla, L., Noormets, M., Moor, U., Karp, K., Jalakas, M. 2011. Effect of rootstock on taste-related properties of Nordic apple cultivars. *Acta Horticulturae* 903: 405–410.

III Mainla, L., Moor, U., Karp, K., Püssa, T. 2011. The effect of genotype and rootstock on polyphenol composition of selected apple cultivars in Estonia. *Žemdirbystė=Agriculture* 98 (1): 63–70.

IV Mainla, L., Moor, U., Karp, K., Tõnutare, T. 2012. The effect of pre-harvest Ca treatment on concentration of polyphenols and antioxidant capacity of 'Pirja' and 'Maikki' apples grown on different rootstocks. *Scientia Horticulturae* 148: 93–96.

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| Paper | Idea and study design | Data collection/ biochemical analyses | Data analysis | Manuscript preparation |
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| I | KK | UM, LM, PP, MS | UM, LM | UM, KK, LM |
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| III | LM, UM | LM, UM | LM, TP | LM, KK, UM |
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LM – Leila Mainla; UM – Ulvi Moor; KK – Kadri Karp; TP – Tõnu Püssa; TT – Tõnu Tõnutare; MN – Merrit Noormets; PP – Priit Põldma; MN - Madli Jalakas; MS –Marge Starast

# ABBREVIATIONS

| | |
|---|---|
| CAT | Catechin |
| CHL | Chlorogenic acid |
| CYGL | Cyanidin glucoside |
| DPPH | 1.1-diphenyl-2-picrylhydrazyl |
| FW | Fresh weight |
| HPLC | High-performance liquid chromatography |
| PHL | Phloridzin |
| SSC | Soluble solids content |
| SSC/TA | Soluble solids and titratable acids ratio |
| TA | Titratable acidity |
| TAC | Total antioxidant capacity |
| QERT | Quercetin |
| QGAL | Quercetin galactoside |
| QTRI | Quercitrin |

# 1. INTRODUCTION

In the past, the agricultural sector was oriented to optimizing yields and producing products with good appearance and shelf –life. Health and other concerns were not at the forefront in selecting crop varieties or production methods (Traill *et al.*, 2008). Winter hardiness of fruit trees has been a very important trait in the temperate zone (Gelvonauskis *et al.*, 2000) and in the Nordic climate it has been an important breeding goal for many years. Nowadays, the aim is to secure a profitable and internationally competitive food and farming sector that respects the environment and improves nutrition and public health (Traill *et al.*, 2008).

Apples are the most popular fruit of temperate climatic zones. Due to high storability, apple is the symbol of convenient fruit available in retail throughout Europe all year round (Konopacka *et al.,* 2010). From 2003 to 2005, the average per capita apple consumption in Europe was 61 g per day, which is twice that of per capita consumption worldwide and represents one-quarter of total European fruit consumption (Ceymann *et al.,* 2012). Earlier studies have shown that the primary factor influencing food choice of European consumers was quality/freshness followed by taste, healthy diet, price, family preferences and habits (Lappalainen *et al.,* 1998). Eating quality is still a key factor driving the choices of consumers in fruit and vegetable consumption (Harker *et al.,* 2003). However, the increased interest in nutrition and healthy eating has led consumers to become more interested in healthier food products (Grunert, 2006). In addition to satisfying traditional sensory and chemical/physical quality criteria, fruits and berries should be high in vitamins and other health-related components, like polyphenolics (Skrede *et al.*, 2012). The study of apple preferences of Estonian consumers showed that both in 2006 and 2011 the main characteristics affecting the decision to purchase apples were (in decreasing order) taste, appearance, health benefits and price (Moor *et al.,* 2013).

Apples are a rich source of phytochemicals, and epidemiological studies have linked the consumption of apples with reduced risk of some cancers, cardiovascular disease, asthma and type II diabetes (Knekt *et al.*, 2002). The antioxidant capacity of apples is mostly attributed to phenolic compounds such as flavonoids and phenolic acids (Lee *et al.*, 2003).

Apple and strawberries were the biggest suppliers of cellular antioxidant activity in USA (Wolfe *et al.*, 2008). Among Spanish consumers, apples were found to be the largest contributor of total flavonoid intake (23.0%) followed by red wine (21.0%) (Zamora-Ros *et al.*, 2010). Similarly in Finland, the main sources of flavonols, flavanones, and flavones were apples, citrus fruit and tea (Ovaskainen *et al.,* 2008).

The distribution of phenolic compounds in fruits varies considerably among different cultivars and also within different tissues (Khanizadeh *et al.,* 2008). The synthesis of these compounds is, in addition to genetic background, influenced by environmental factors such as nutrient availability, temperature, light (Saure, 1990; Treutter, 2001; Hagen *et al.*, 2007), cultivation methods such as canopy pruning and fertilization, and rootstock (Awad and de Jager, 2002; Scalzo *et al.,* 2005).

At present, there are literature reports on the contents of selected apple polyphenols in a small number of cultivars (Ceymann *et al.,* 2012). Data about phenolic content in Baltic apple cultivars is almost non-existent. Information about the effect of rootstock on polyphenol content is also very rare. This thesis aims to give the first insight into polyphenol composition of different Baltic apple cultivars as well as discuss the possible interactions between apple nutrition and rootstock and its effect on apple fruit composition.

# 2. EFFECT OF AGRICULTURAL PRACTICES ON APPLE COMPOSITION AND STORABILITY

## 2.1 Apple biochemical composition and storability as affected by foliar Ca treatment

In spite of a high Ca content in most orchard soils and a high potential of Ca uptake in apple trees, there is no doubt that Ca deficiency is a causal factor for many disorders in apple fruits (Vang-Petersen, 1980). Bitter pit and other physiological disorders are associated with low Ca concentrations in the fruit and pre-harvest Ca treatment is a common agricultural practice to increase the concentration of Ca in apples for better storability (Dris and Niskanen, 1998; Kadir, 2004; Saure, 2005; Val *et al.*, 2008). Calcium nitrate and calcium chloride are commonly used fertilizers for foliar fertilization (Casero *et al.*, 2010). However, the recommendations on spray times, frequency and concentration vary greatly between different regions, which lead to inconsistent results. Because the effectiveness of Ca sprays is influenced by environmental conditions and cultivar (Ernani *et al.*, 2002; Val *et al.*, 2008), it is important to develop pre-harvest Ca-treatment schemes suitable for our region.

The most abundant mineral elements in apples are Ca, N, K, P and Mg (Nachtigall and Dechen, 2006). Adequate Ca content in apple tissue is necessary to obtain high quality fruit (Conway *et al.*, 2002). Excessive amount of N in apples can inhibit the assimilation of other elements, especially K and Ca (Lamp, 1995). Ca deficiency and N excess in apples causes physiological disorders during storage. Low concentration of K determines some problems in apple quality, especially during storage (Campeanu *et al.*, 2009). However, apple quality is not only determined by concentration of specific mineral elements, but also by their ratios (Casero *et al.*, 2010). For good quality apples, the N/Ca ratio should be either about 10 (Shear, 1974) or ranging from 10 to 14 (Sharples, 1980; Dris, 1998). In bitter pit free fruits the Ca levels were higher and N/Ca and K/Ca ratios decreased (Fallahi *et al.*, 2006). The equilibrium between N and P determines the accumulation of sugars and quality of the fruit (Campeanu *et al.*, 2009). An increase in N, P, and K content in relation to Ca appears to hasten the metabolic processes that lead to the softening of the fruit (Casero *et al.*, 2010). Since the mineral content of apples is also influenced by climatic and soil factors, fruit growing conditions and farming practices (Lamp, 1995; Dris and Niskanen, 1998; Saure, 2005; Casero *et al.*, 2010), the influence of pre-harvest Ca treatment on apple mineral content is variable. Varying climates or growing conditions lead to differences in the accumulation of minerals other than Ca. An imbalance between mineral nutrients with respect to Ca content should therefore be taken into account when fruit quality is assessed (Dris *et al.*, 1998; Fallahi *et al.*, 2006; Casero *et al.*, 2010).

Ca treatment has an increasing (Yuri *et al.*, 2002; Farag and Nagy, 2012), decreasing (Dris and Niskanen, 1999; Baneh *et al.*, 2003) or no influence (Wojcik, 2002; Kadir, 2004) on apple soluble solids content (SSC). The same tendency can be found with titratable acidity (TA). The effect of foliar fertilization depends on the fertilizer used. The pre-harvest study provided evidence that $CaCl_2$ alone or combined with $MgCl_2$ resulted in the firmest apple fruits followed by $MgSO_4$ plus $CaSO_4$ (Farag and Nagy, 2012). Moreover, the highest values of SSC were found in fruits treated with $CaSO_4$ alone or combined with $MgSO_4$ when compared with untreated fruits (Farag and Nagy, 2012). Hence, the effect of fertilizers is different. Because the sugar and acids contents of apples are depending on the weather conditions, cultivars, culture technology, position and exposition of the fruits in the crown (Mitre *et al.*, 2009; Sestras *et al.*, 2009). High contents of sugar are obtained, due to weather conditions with high temperature and high nutritive element contents of soils, which permit the assimilation of sugars (Campeanu *et al.*, 2009). Moreover, apple SSC and TA have been found to be correlated to fruit mineral content. Increase in N and K concentration in fruit flesh increases fruit TA at harvest and after storage as well as SSC at harvest (Casero *et al.*, 2010). Studies in fruit quality have indicated relationships between °Brix levels and TA ratio and consumer acceptability (Vangdal, 1985; Harker *et al.*, 2002). As apples ripen, their content of TA decreases. However, in overripe apples the concentration of TA has decreased too much and the soluble solids content and titratable acids ratio (SSC/TA) increases. The ratio over 20 significantly decreases the taste-properties of apples (Kelt, 1981).

Consumers are also interested in the health benefits of apples. Studies *in vitro* and *in vivo* have shown the beneficial effect of fruits and vegetables on human health (Wang *et al.*, 1997; Joshipura *et al.*, 1999). Much of the protective effect has been attributed to phytochemicals, which are non-nutrient plant compounds such as carotenoids, flavonoids, isoflavonoids and phenolic acids (Kampa *et al.*, 2004). The synthesis of these compounds is, in addition to genetic background, influenced by environmental factors such as nutrient availability, temperature, light (Saure, 1990; Treutter, 2001; Hagen *et al.*, 2007), cultivation methods such as canopy pruning and fertilization, and rootstock (Awad and de Jager, 2002; Scalzo *et al.*, 2005). Total antioxidant capacity (TAC) varies among fruits of different species and is strongly influenced by genotype (species or cultivar) (Scalzo *et al.*, 2005). The distribution of phenolic compounds varies considerably among different cultivars and also within different tissues (Khanizadeh *et al.*, 2008). Phenolic compounds are induced in plants by various biotic and abiotic stresses (Dixon and Paiva, 1995). Cold treatments and drought stress cause increases in levels of (-)-epicatechin and quercetin-3-galactoside in *Crataegus laevigata* and *C. monogyna*. These types of treatments also enhance the antioxidant capacity of the shoot extracts, and may be the primary function of these cold-inducible flavonoids (Kirakosyan *et al.*, 2003). Marais *et al.* (2001) have found that a fluctuating temperature resulted in better colour and higher anthocyanin concentrations for apple fruits harvested from different areas. A positive effect of pre-harvest Ca treatment on apple phenolics was observed by Sannomaru *et al.* (1998), who found that the content of epicatechin, chlorogenic acid (CHL) and total polyphenol of 'Starking' apples was higher in Ca treated than in untreated fruit. Awad and de Jager (2002) found an occasional positive correlation between Ca and total flavonoids in apple skin. They also stated that the most important variable in predictive models for the anthocyanin and total flavonoids concentration was N concentration in the fruit. The concentrations of cyanidin 3-galactoside (anthocyanin), catechin (CAT) and total flavonoids were generally decreased by increasing the amount of N fertilizer (Awad and de Jager, 2002). It appears that among mineral elements, Ca and N play key-roles in apple quality.

## 2.2. Apple biochemical composition as affected by rootstock

The establishment of intensive apple orchards in Estonia has been delayed due to missing information concerning suitability of particular dwarfing rootstocks for Estonian conditions (Univer *et al.*, 2006). Orchards with dwarfing and semi-dwarfing clonal rootstocks are also of interest to farmers in other Baltic states (Rubauskis and Skrivele, 2007; Kviklys *et al.*, 2012).

Influence of rootstock on tree performance depends on many factors: soil, climate, moisture, orchard management etc. (Autio *et al.*, 2001; Kviklys *et al.*, 2006; Haak, 2007; Univer *et al.,* 2010; Univer *et al.*, 2013). Grafting rootstocks are widely used to enhance plant resistance to various biological and abiotic stresses (Liu *et al.*, 2012). Previous results suggest that the choice of rootstock can enhance drought resistance by improving the antioxidant system in an apple tree (Liu *et al.*, 2012). In Estonian conditions, M.26 is considered to be semi-dwarfing, M.9 and B.396 as dwarfing rootstocks (Haak, 2007; Univer *et al.*, 2013). However, compared to M.26 and B.396 M.9 is not considered to be winter hardy (Univer *et al.*, 2013).

Rootstocks have a significant influence on the intake of mineral elements from the soil. Studies have shown that the content of macronutrients in the leaves depends on the rootstock (Poniedziałek *et al.*, 1993). Sotiropoulos (2008) found a higher concentration of N in the leaves of 'Imperial Double Red Delicious' grown on the seedling compared to the rootstocks M.7 and MM.106. Leaf Ca decreased but leaf K increased with rootstock vigour, resulting in the greatest leaf Ca but lowest leaf K in trees on 'Budagovsky 9' (Fallahi, 2012). Rootstock also affects the accumulation of Ca in the fruit (Gąstoł and Poniedziałek, 2005).

In rootstock/cultivar combination the tree growth and yield quality is influenced by both components (Skrzynski, 2007; Rubauskis and Skrivele, 2007). Yields per tree tend to be closely related to tree size: rootstocks inducing the largest trees induced also the highest yield (Univer *et al.*, 2010; Kviklys *et al.*, 2012). However, the effect of rootstock on apple yield was clearly modified by the effect of location (Kviklys *et al.*, 2012). The influence of rootstocks on apple SSC and TA content differ with year and depends on the rootstock type (Daugaard and Callesen, 2002; Skrzynski and Gąstoł, 2006). Kviklys and Kvikliene (2002) found that fruits from trees on low-vigour rootstocks, such as P 22, M.9 or P 2, contained much more SSC than fruits from trees grown on more vigorous, M.26 or P 60 rootstocks.

According to the reported data the important factors influencing the content of polyphenols in apples are: cultivar properties, fruit maturity, weather conditions of the harvesting season, processing, agricultural conditions, crop load, development of infection, fruit position within the canopy and geographic location (Awad *et al.*, 2000; Van der Sluis *et al.*, 2001). On the other hand, considering factors influencing apple quality, the list also includes rootstocks. From the health point of view the concentration of polyphenols is considered to be an important component of apple quality. Hence, the influence of rootstock should also be taken into account as an important factor influencing the concentration of polyphenols.

Scalzo *et al.* (2005) have found that the rootstocks in apricots and peaches play an important role in determining the total amount of phenolic compounds. In apricots and peaches, the TAC was also influenced by the rootstock (Scalzo *et al.*, 2005). Compared to seedlings, the dwarfing effect of vegetative rootstocks might have a beneficial influence on the concentrations of polyphenols, because canopies with weaker shoot growth provide better light conditions for fruits. Fruits from dwarf and high yielding trees showed the highest percentage of fruit surface covered by red colour (Kviklys *et al.*, 2012). A tendency to poorer coloration was noted with increasing rootstock vigour (Kviklys *et al.*, 2012). According to Hagen *et al.* (2007) the concentration of flavonoids and the level of total phenols are higher in the peel of sun-exposed apples compared to shade-grown apples.

# 3. HYPOTHESES AND AIMS OF THE STUDY

Pre-harvest Ca treatment is widely used in many parts of the world to reduce or prevent the incidence of physiological disorders during storage. However, the information concerns bitter pit more than other physiological disorders.

Several reports state that pre-harvest foliar Ca treatment and rootstocks have an effect on different apple quality parameters. However, the information about the effect of rootstock and pre-harvest Ca treatment on apple polyphenol content is rare.

Hypotheses of the present study:

- mineral nutrition of apples could affect other physiological disorders besides bitter pit;
- by using foliar Ca treatment or different rootstock/cultivar combinations, it is possible to affect apple taste-related properties and polyphenol concentration and, through that, also the total antioxidant capacity.

The aims of the present study were to find out:

- the influence of pre-harvest Ca treatment on apple mineral composition and different physiological disorders (**Paper I**);
- the effect of pre-harvest Ca treatment on different rootstocks on apple taste-related properties, polyphenol concentrations and total antioxidant capacity (**Paper IV**);
- the effect of rootstocks on apple mineral composition, taste-related properties and polyphenol concentrations (**Papers II**, **IV**);
- the influence of genotype on apple polyphenol composition (**Paper III**).

# 4. MATERIAL AND METHODS

## 4.1. Plant material and agricultural practices

Foliar Ca treatment experiments with ‘Krameri tuviõun’ and ‘Talvenauding’ were conducted in a commercial orchard of the company Vasula Aed, which is situated in Tartu County, South Estonia (58°28’ N, 26°44’ E) (Table 1). Apple trees were planted in 1975 with a distance of about 8m between rows and 4 m within the row. No irrigation system was used in the plantation.

The experiment began in June 2002 and finished in April 2004. The following Estonian apple cultivars were used for the experiment: ‘Krameri tuviõun’: fruit weight 80–110g, ripens in November and is meant for storage until January. ‘Talvenauding’: fruit weight 80–125g, ripens in November and is meant for storage until March. ‘Antonovka’ was used as seedling rootstock for all cultivars.

The soil in the experimental area is a sandy loam, Endoeutri – Haplic Luvisol. The depth of the humus horizon ranges from 24 to 30 cm. Soil samples were taken in September using a steel auger at depths of 20-50 cm. The samples comprised of a mixture of 10 subsamples from both the control trees (unfertilised) and calcium-treated trees areas. The following elements were determined from soil samples: P- (ammonium lactate extractable), K- (ammonium lactate extractable), Ca- and Mg- (1 M ammonium acetate extract, pH 7.0). Soil analyses indicated that there was no deficiency of any nutrients in the soil.

Foliar fertilization of apple trees was applied as follows: in 2002, $Ca(NO_3)_2$ 1% solution was applied on 9 and 30 July at a rate of 660 L ha-1, and $CaCl_2$ 0.5% solution was applied on 22 August at the same rate. In 2003, $Ca(NO_3)_2$ was applied on 14 and 28 July and $CaCl_2$ on 8 August and 1 September.

Apples were harvested during the second and third week of September in 2002 and 2003, respectively. Samples of 300 fruits per plot (fifty fruits in six replications) were collected at random. First quality apples were picked according to an equatorial pattern (North-South-East-West) from the outside of the trees avoiding fruit situated at the top, the bottom and also deep inside the canopy. Apple fruits were stored in a commercial coolstore of the company Vasula Aed at 2–5°C and 80–85% relative humidity (RH). Fifty fruits were placed on one layer in air-permeable plastic boxes. The storage period for 'Krameri tuviõun' was 4 months and for 'Talvenauding' 6 months.

**Table 1.** Overview of conducted experiments.

| Impact factor | Experiment subject | Analyzed parameters | Year | Plantation location |
|---|---|---|---|---|
| Foliar Ca fertilizers $CaCl_2$ and $Ca(NO_3)_2$ | 'Krameri tuviõun', 'Talvenauding' Seedling ('Antonovka') | Ca, N, P, K, Mg, superficial scald, bitter pit, physiological spot | 2002-2004 | Company Vasula Aed |
| Foliar Ca fertilizer $CaCl_2$ | 'Pirja', 'Maikki' rootstocks M.26, B.396 | Ca, N, SSC, TA, SSC/TA, CAT, CHL, PHL, QERT, QGAL, QTRI, CYGL, TAC | 2008 | Rõhu Research Center |
| Rootstocks M.26, M.9, B.396 | 'Valge klaarõun', 'Maikki', 'Pirja', 'Krasnoje rannjeje' | TA, SSC, SSC/TA | 2003 2004 | |
| Rootstocks M.26, B.396, seedling ('Antonovka') | 'Talvenauding' | CAT, CHL, PHL, QERT, QGAL, QTRI | 2006 2007 | |
| Genotype | 'Talvenauding', 'Krista', 'Liivi kuldrenett', 'Lobo', 'Cortland', 'Antei' Seedling ('Antonovka') | CAT, CHL, PHL, QERT, QGAL, QTRI | 2006 2007 | |

CAT – catechin, CHL – chlorogenic acid, PHL – phloridzin, QERT – quercetin, QGAL – quercetin galactoside, QTRI – quercitrin, CYGL – cyanidin glucoside, SSC – soluble solids, TA – titratable acidity, SSC/TA – soluble solids and titratable acidity ratio.

The rootstock and Ca treatment experiments with summer cultivars were carried out in South Estonia at the Estonian University of Life Sciences' Rõhu Research Center (58°21' N, 26°31' E) (Table 1). The trees were planted in spring 2001 with a distance of 2 m between the trees and 4 m between the rows. Trees were in randomized complete block design with four replicates and four trees per plot. The trees were trained as a spindle and were not irrigated. The ground between the rows was grassed and along the rows treated with herbicides. Plants were only fertilized in 2001 as followed (kg ha$^{-1}$): N 20, P 40 and K 40.

The rootstock experiment with four summer cultivars 'Valge klaarõun', 'Maikki', 'Pirja' and 'Krasnoje rannjeje' on rootstocks M.26, M.9 and B.396 was carried out in 2003 and 2004. 'Valge klaarõun' on M.9 had no apples in 2003 therefore it was excluded in the first year.

The rootstock and Ca treatment experiment with summer cultivars 'Pirja' and 'Maikki' on M.26 and B.396 was carried out in 2008. The original plan was to repeat the experiment in 2009, but since there was an enormous amount of snow in winter, the plantation suffered from flooding in the spring, which caused abnormal growth of leaves and shoots because of water stress. Several trees later died and had to be replaced. Under these circumstances the experiment could not be repeated. The following foliar Ca treatments were used: 1. Control (no Ca treatment); 2. $CaCl_2$ (0.5% $CaCl_2$ solution, 1000 L ha$^{-1}$) sprayed onto the trees on 22 June, 3 and 15 July.

'Pirja' apples were harvested on 21 July and 'Maikki' on 6 August. Harvest maturity was determined by seed colour (the tips of the seeds turned brown) and confirmed by the Iodine-starch test (a narrow area of the fruit flesh (2 to 3 mm) under the skin turned blue). Apples were picked according to an equatorial pattern (North-South-East-West) from the outside of the trees avoiding fruit situated at the top, the bottom and also deep inside the canopy. The yield was harvested from all experimental trees. From the total yield three samples (3 x 5kg fruits) was randomly taken for analyses. For determination of polyphenols, mineral elements and TAC, ten fruits per replicate were selected randomly for each analysis. Before the analyses, apples were cooled for 24 h in a cool store at 6°C.

The experiments on influence of genotype and rootstock on apple polyphenol concentration were carried out in South Estonia at the Estonian University of Life Sciences' Rõhu Research Center (Table 1). The effect of genotype on polyphenol concentration of 'Talvenauding', 'Krista', 'Liivi kuldrenett', 'Lobo', 'Cortland', and 'Antei' apples was studied in 2006 and 2007. The apple trees were grafted onto 'Antonovka' seedlings and planted in 1986 with a distance of 4 m between the trees and 8 m between the rows. The effect of rootstock on apple polyphenol concentration of 'Talvenauding' grafted onto semi-dwarfing rootstock M.26, dwarfing rootstock B.396 and vigorous rootstock 'Antonovka' seedling was studied in 2006 and 2007. The trees were planted in 2001 with 2 m between the trees and 4 m between the rows.

The ground between the rows was grassed and the rows were treated with herbicides. Soil analysis of the vegetative rootstock experiment orchard showed: $pH_{KCl}$ 7.0, P – 175 mg $kg^{-1}$, K – 197 mg $kg^{-1}$, Ca – 2980 mg $kg^{-1}$ and humus – 4.7%. Soil analyses for the cultivar testing orchard showed: $pH_{KCl}$ 6.3, P – 216 mg $kg^{-1}$, K – 236 mg $kg^{-1}$, Ca – 2830 mg $kg^{-1}$ and humus 5.9%. Since the soil analyses showed no nutrient deficiencies and the trees were growing well, no mineral fertilizers were used at the experimental plantation in either of the experimental years. For plant protection, the apple trees were sprayed four times during the vegetation period to prevent apple scab using copper oxide chloride at the end of April, cypprodinil in the middle of May, difenoconazole at the end of June and ditianon in the middle of July.

In both years, 'Krista' and 'Liivi kuldrenett' apples were harvested in mid-September, 'Antei', 'Talvenauding', 'Cortland' and 'Lobo' in the third ten-day period of September. Samples of 50 first quality fruits per cultivar were picked from the outer periphery of the canopy. Fruits were stored at between 2 and 5°C and 90…95% RH in a normal atmosphere storehouse for three months. The analyses of polyphenols were performed on the ripe apples at the end of November.

### 4.2. Weather conditions

According to agro climatic regions of Estonia, and also taking into account several studies of microclimate, Kask (2000) has divided Estonia into different regions based on their suitability for fruit production. Rõhu and Vasula are situated in the Tartu region, where cold winters with very low temperatures (sometimes below –37 °C, in certain areas –39 °C) are typical. A vegetation period free of night frosts is short (130 days) and night frosts may be severe. Snow falls quite late and severe frosts in November without snow cover (below –20°C or even more on the ground) may damage fruit crops.

Summer 2002 was warmer with less precipitation than the long-term average in Estonia. The mean air temperature was especially higher than the average for July and August (**Paper I** Table 2). At the same time precipitation was extremely low in May, when only 15.4 mm of rain fell, compared to an average of 55 mm for this time period. Precipitation was also very low in July, August and September. In 2003, the air temperature of July was approximately the same as the long-term average (18 °C). June (13 °C) and August (15 °C) were cooler than the average (15 °C and 17 °C respectively). At the same time summer was very wet; especially a large amount of rain fell in May (143 mm), July (104 mm) and August (133 mm). In 2004, the air temperature in August was approximately the same as the long-term average. Both June and July were cooler than the average. July (76 mm) was drier than the average (80 mm), but June (184 mm) and August (105 mm) were very wet. Especially in June, when there was 2.5 times more rain than usual at this time.

In 2006, the weather during May, June and July was fairly dry as only 49% of the average precipitation for these three months fell (**Paper III** Fig. 1). Only in August 2006, the precipitation was more than the average for this period. Conversely in 2007, the precipitation for May was double the average and the driest month was August. In 2006, the mean air temperature was above the average, being especially high in July: 18.5 °C, (average 16.7 °C) and September 13.1 °C (average 10.4 °C). The mean air temperature in 2007 was close to the average except for May 13.6 °C (average 11.0 °C) and August 17.7 °C (average 15.6 °C). The duration of sunlight hours in 2006 was longer in July and September and shorter in August than the average, but in 2007 was longer in June and August and shorter in July and September compared to the average.

### 4.3. Measurements and analyses

Spoiled apples were removed from healthy apples monthly and divided into categories, which were 'Talvenauding' fruits with superficial scald and other quality loss symptoms and 'Krameri tuviõun' fruits with calcium deficiency symptoms (bitter pit), physiological spot, and other symptoms (**Paper I**).

The fruit content of Ca, N, P, K and Mg was determined after harvest. N concentration of air-dried samples was determined by the Kjeldahl method. The method involves digestion of the sample in sulphuric acid using the Kjeldahl Cu catalyst to convert the protein nitrogen to ammonium sulphate. Ammonia is then liberated by alkaline distillation using the automatic analyser Kjeltec Auto 1030. P and Mg concentrations were measured from a Kjeldahl digest using the flow injection analyzer "FIAstar 5000", K concentration was determined flamephotometrically by an air-acetylene flame. P was determined at a wavelength of 720 nm by the Stannous Chloride method. Mg was determined by the Titan Yellow at a wavelength of 540 nm. Fruit Ca was determined by an induction couplet plasma spectrometer. All nutrient concentrations were expressed as mg $kg^{-1}$ fresh weight (FW) (**Papers I** and **IV**).

Ten fruits from each cultivar from each treatment and replicate were taken for determination of TA and SSC. Apples were sliced, core removed and both skin and flesh tissue were used for all analyses. TA was determined by taking 5 g from pounded samples, put into 100 ml bulbs, which were filled with 80 °C distilled water. The dilutions were heated for two hours in 80 °C, then cooled down to the room temperature, filtered and titrated with 0.1N NaOH until pH 8.2. TA was expressed as g 100 $g^{-1}$ FW malic acid. SSC was measured using digital refractometer (ATAGO CO.). SSC was expressed as °Brix (**Paper II**).

Catechin (CAT), chlorogenic acid (3-O-caffeoylquinic, CHL), quercitrin (quercetin-3-rhamnoside, QTRI), quercetin-3-galactoside (QGAL), quercetin (QERT), phloridzin (phloretin-2-O-glucoside, PHL) and cyanidin-3-glucoside (CYGL) in the apple peel extract were identified by their chromatographic retention times and $MS^2$ fragmentation spectra when compared to the respective parameters of commercial standards. Standard chemicals for identifying and quantifying different polyphenols as well as formic acid were sourced from "Sigma-Aldrich Buchs" Co. Methanol and HPLC (high-performance liquid chromatography) grade were obtained from Rathburn Chemicals Ltd. Six more polyphenols were putatively identified, but not quantified, by their fragmentation spectra. Sample UV-chromatograms at two wavelengths are presented in Figure 2 in **Paper III**. (-)-epicatechin was not quantified because of the same retention time with cyanidin-3-glucoside (**Papers III** and **IV**).

Peel (approximately 1 mm thick and weighing 0.2 g) at room temperature was cut from ten apples in three replications per cultivar, and rapidly transferred into a tube with 1 ml 0.01 M HCl in methanol. The samples were shaken for 30 minutes at 40 rpm using "Biosan Multi RS60". After shaking, the extract was transferred to another tube before second and third re-extractions of the peel using the same procedure as described above. The total extract (3 ml) was centrifuged by "Eppendorf Centrifuge 5810R" ("Eppendorf" AG) for 15 minutes at 4000 rpm. The extract was then cooled to 15°C and 1 ml of extract was filtered through 0.45 µm "Millex-FH" filter and sealed into an airtight glass capsule ("Agilent"). "Agilent 1100 Series HPLC" device equipped with a reversed phase Zorbax 300SB-C18 column (2.1×150 mm, 5 µm particle size – "Agilent"), photodiode array detector and an electrospray ionization ion trap MS/MS detector ("1100 Series LC/MSD Trap-XCT", "Agilent Technologies"), operated in negative mode ionization (m/z interval 100–1000 amu, target mass – 400 amu) was used for identification and quantification of polyphenols from the filtered peel extract. The column was eluted at 0.3 ml per minute with a gradient of 0.1% aqueous formic acid (solvent A) and of acetonitrile (solvent B) from 5 to 95%. The HPLC 2D ChemStation software with a ChemStation Spectral SW module ("Agilent Technologies") was used for process guidance and primary processing of the results. The concentration of polyphenols in the apple peel was calculated according to respective calibration curves obtained using standard compounds and expressed as mg 100 $g^{-1}$ FW. The estimated content of total polyphenols was calculated as the areas under chromatographic curves (AUC) at $\lambda$ = 280 nm (**Papers III** and **IV**).

For determination of TAC, ethanolic apple extract was used in the 1.1-diphenyl-2-picrylhydrazyl (DPPH) discoloration assay described by Brand-Williams *et al.* (1995) with some modifications. An aliquot of apple extract at different concentrations was added to the DPPH solution and the absorbance at 515 nm was measured for 120 minutes (until the reaction had reached a plateau). The EC50 parameter, the amount of sample necessary to decrease by 50% the initial DPPH concentration, was calculated for the apple extracts. For the calibration curve ascorbic acid solutions at different concentrations were used. The results of TAC were expressed as mg ascorbic acid equivalent (AAE) 100 $g^{-1}$ FW as suggested by Kim *et al.* (2002) (**Paper IV**).

### 4.4. Statistical analyses

One-way ANOVA were applied to test the effect of various factors (fertilization, rootstock and cultivar) on the apple biochemical parameters. Fisher's least significant difference (LSD) test for homogeneous groups was used for testing significance between experimental treatments. The level of statistical significance was set at $P \leq 0.05$. In figures and tables the mean values to be compared are followed by the same letter if they are not significantly different at $P \leq 0.05$ (**Papers I–IV**).

To study correlation between fruit mineral nutrient content and fruit spoilage, disorders, polyphenols content, correlation analysis was used. Linear correlation coefficients between variables were calculated, the significance of coefficients being $P \leq 0.001$***, $P \leq 0.01$**, $P \leq 0.05$*, ns = non-significant (**Papers I** and **IV**). Regression analysis was used to determine the relationship between the fruit polyphenols and total antioxidant capacity. The coefficient of determination ($R^2$) was calculated (**Paper IV**).

# 5. RESULTS

## 5.1. Effect of Ca treatment on the composition and postharvest quality of apples

### 5.1.1. Mineral composition

The pre-harvest Ca sprays ($CaCl_2$ and $Ca(NO_3)_2$) had a significant influence on the mineral composition of 'Talvenauding' and 'Krameri tuviõun' apples (**Paper I** Fig.2). In the first experimental year (2002), Ca content of 'Talvenauding' and 'Krameri tuviõun' fruits ranged from 35 to 46 mg $kg^{-1}$ FW, N content from 264 to 460 mg $kg^{-1}$ FW, K content from 1087 to 1471 mg $kg^{-1}$ FW and P content from 35 to 53 mg $kg^{-1}$ FW. Ca treatment significantly influenced mineral composition of both cultivars, but differently. The treatment significantly increased N content in 'Krameri tuviõun', but did not influence N content in 'Talvenauding' fruits. Fruit K content was increased in 'Krameri tuviõun' but decreased in 'Talvenauding'. Ca sprays increased P content in fruits of both cultivars. Mg content ranged from 9.9 to 12.5 mg $kg^{-1}$ FW and was not influenced by Ca treatment (data not shown).

In the second experimental year (2003), fruit Ca content ranged from 30 to 65 mg $kg^{-1}$ FW, N content from 343 to 397 mg $kg^{-1}$ FW, P content from 91 to 169 mg $kg^{-1}$ and Mg content from 50.9 to 71.9 mg $kg^{-1}$ FW (**Paper I** Fig. 2). Ca content of treated 'Talvenauding' increased significantly, being 60% higher than in 'Krameri tuviõun'. Ca treatment significantly decreased N content in 'Krameri tuviõun' apples. K content ranged from 1115 to 1748 mg $kg^{-1}$ FW and was not affected by the treatment (data not shown). Ca sprays significantly increased Mg content in 'Talvenauding' fruits, but had no effect on 'Krameri tuviõun'. Ca treatment reduced significantly P content in 'Krameri tuviõun'.

The N/Ca in apples was similar in both years, remaining between 6 and 13 (**Paper I** Fig. 3). In 2002, Ca treatment increased N/Ca in 'Krameri tuviõun': N/Ca in apples was almost double that in 'Talvenauding'. In 2003, Ca sprays affected both cultivars, decreasing the N/Ca in apples by an average of 31%. Similar to the previous year, the N/Ca in 'Talvenauding' was lower. The average K/Ca ratio in apples was 30.9 and 34.5 in 2002 and 2003, respectively. In both years, Ca treatment significantly reduced the K/Ca in 'Talvenauding'. The Ca treatment had an increasing effect on K/Ca in 'Krameri tuviõun' in 2002, and no effect in 2003. The Mg/Ca in apples differed yearly, ranging from 0.20 to 0.35 in 2002 and from 1.0 to 1.8 in 2003. The Mg/Ca of 'Krameri tuviõun' was not affected by Ca treatment in either year, while the Mg/Ca in 'Talvenauding' in 2002 was increased and in 2003 decreased by the treatment.

### 5.1.2. Postharvest loss and physiological disorders, correlations with mineral composition

The effect of pre-harvest Ca sprays ($CaCl_2$ and $Ca(NO_3)_2$) on 'Krameri tuviõun' and 'Talvenauding' apples storability and incidence of physiological disorders (superficial scald, bitter pit, physiological spot) was studied in 2002 and 2003 (**Paper I** Fig.1). In 2002, Ca treatment did not affect postharvest loss in either cultivar (data not shown). In 2003, first signs of spoiling were noticed in 'Krameri tuviõun' after two months of storage. Ca treatment significantly reduced postharvest loss. The effect remained significant until the end of storage, decreasing the number of spoiled apples of 'Krameri tuviõun' by 33%. After four months of storage, fruits of 'Talvenauding' started to suffer from superficial scald. The effect of Ca treatment was significant, decreasing the number of spoiled apples by 57%. At the end of the storage period the effect of Ca treatment on spoilage of 'Talvenauding' fruits was no longer significant.

Bitter pit incidence was not affected by Ca treatment in either experimental year. Physiological spot was significantly decreased by Ca sprays in 2003. Superficial scald of 'Talvenauding' fruits was also significantly decreased by the treatment in 2003.

In both experimental years, the percentage of spoiled fruits after four months of storage correlated negatively with fruit Ca content and positively with N/Ca balance in the fruits (**Paper I** Table 3). In the second year, fruit spoilage after four months of storage had a significant negative correlation with fruit K, Mg and P content.

Regarding the different physiological disorders, correlations were different from general correlations with loss of marketable yield (**Paper I** Table 3). As an average of two years observations, superficial scald of 'Talvenauding' correlated negatively with Ca content and positively with K/Ca, N/Ca and Mg/Ca. Bitter pit in 'Krameri tuviõun' correlated positively with Ca content and negatively with Mg and P content and Mg/Ca in apples. Physiological spot on the same cultivar had a significant positive correlation with apple K and N content and a negative correlation with Mg and P content and Mg/Ca in apples.

### 5.1.3. Taste–related properties

Pre-harvest Ca sprays ($CaCl_2$) had no significant influence on SSC of 'Pirja' and 'Maikki' apples grown on M.26 and B.396. SSC ranged from 9.3 to 10.2 °Brix (data not shown). TA ranged from 0.57 to 0.96 g 100 g$^{-1}$ FW and was significantly affected by the treatment (Fig. 1). In both cultivars, on M.26, fruit TA was decreased by the Ca sprays. In 'Maikki' on B.396, the Ca treatment increased the TA concentration in fruits.

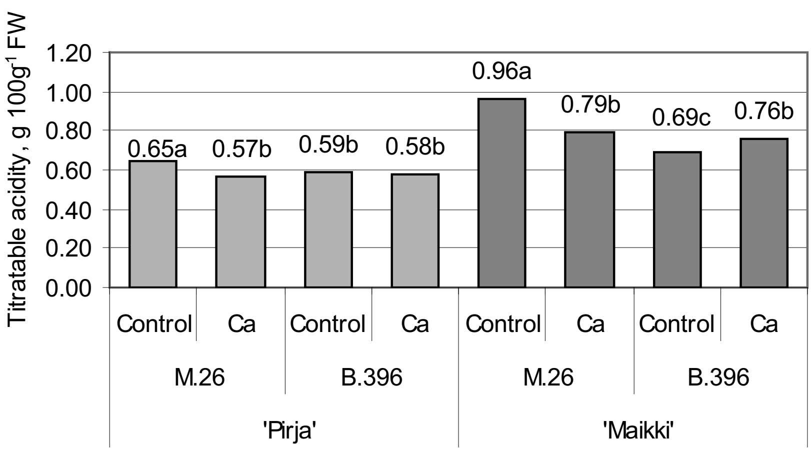


**Figure 1.** The effect of Ca treatment on apple titratable acidity. Means followed by different letters (within cultivar) are significantly different ($P \leq 0.05$).

In both cultivars, SSC/TA was significantly affected by the Ca treatment only when grown on rootstock M.26: the ratio was increased (Fig. 2).

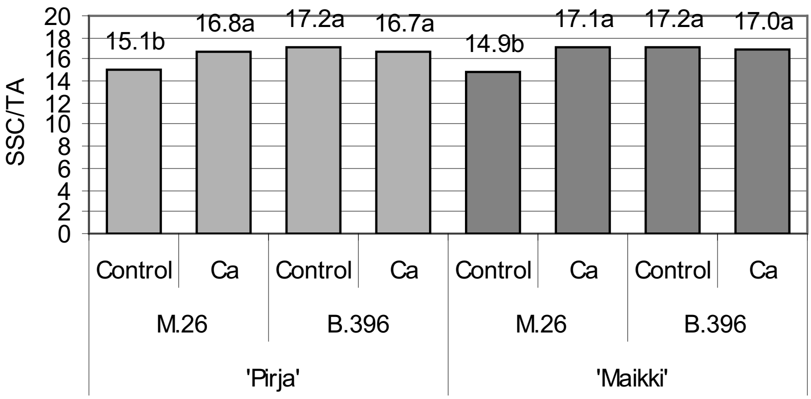


**Figure 2.** The effect of Ca treatment on apple soluble solids and titratable acidity's ratio (SSC/TA). Means followed by different letters (within cultivar) are significantly different ($P \leq 0.05$).

### 5.1.4. Polyphenol composition and antioxidant capacity

The pre-harvest Ca treatment ($CaCl_2$) had a significant influence on polyphenol concentration of 'Pirja' and 'Maikki' apples, but the influence was dependent on the rootstock (M.26 and B.396) (**Paper IV** Table 1). On B.396, the Ca treatment significantly increased the concentration of QTRI in both cultivars: in 'Pirja', the increase was 23% and in 'Maikki' 20%. In 'Maikki' on B.396, the Ca treatment also increased the concentration of CAT, CHL and PHL. On M.26, the Ca treatment significantly decreased the concentration of QTRI in both cultivars, causing a decrease of 43% in 'Maikki' and 20% in 'Pirja'. In the latter cultivar on M.26, the Ca treatment also significantly decreased the concentration of PHL and QGAL and increased only the concentration of CYGL. In 'Maikki' on M.26, the Ca treatment also significantly decreased QGAL and CYGL concentrations.

The correlation analysis indicated that in 'Maikki', Ca had positive significant correlations with QERT, QGAL, QTRI and PHL (**Paper IV** Table 2). N showed negative correlation with QERT, CAT, CHL and PHL. N/Ca also had negative correlations with QERT, QTRI, CAT and PHL.

The TAC varied between the cultivars: ranging from 0.454 to 0.851 mg ascorbic acid 100 $g^{-1}$ FW in 'Pirja' and from 0.299 to 0.521 mg ascorbic acid 100 $g^{-1}$ FW in 'Maikki' (**Paper IV** Fig. 1). In both cultivars, growing technologies caused almost a double variation of TAC. In 'Maikki', a significant positive correlation between TAC and fruit Ca content was found and a negative correlation with N/Ca (**Paper IV** Table 2). In 'Pirja', no significant correlations between Ca content and most of the polyphenols were found. Also, TAC was not correlated with the mineral composition of 'Pirja' (data not shown).

In 'Maikki', significant nonlinear correlations between TAC and the majority of the polyphenols were found: PHL, QERT, QGAL, QTRI, CYGL and CAT (**Paper IV** Fig.1). Only CHL did not have significant correlation with TAC. In 'Pirja', only QTRI and PHL showed significant nonlinear correlations with TAC. The trendline showed an increase of TAC with increasing concentration of QTRI, but with increasing PHL concentration in apples the increase of TAC was slower.

## 5.2. Effect of rootstock on apple mineral composition, taste-related properties and polyphenols

### 5.2.1. Mineral composition

The rootstock (M.26 and B.396) had a significant effect on the mineral content of 'Pirja' and 'Maikki' apples, but the effect was more pronounced in 'Maikki' (Table 2). In 'Pirja', the rootstock had a significant influence only on the content of N and N/Ca: on B.396 the content was significantly higher. In 'Maikki' on B.396, apples had significantly higher content of N, Mg, N/Ca and Mg/Ca. Only the content of Ca was significantly higher on M.26.

**Table 2.** The effect of rootstock on the content of Ca, N, Mg, N/Ca and Mg/Ca in 'Pirja' and 'Maikki' apples.

| Rootstock | Ca (mg kg$^{-1}$ FW) | N (mg kg$^{-1}$ FW) | Mg (mg kg$^{-1}$ FW) | N/Ca | Mg/Ca |
|---|---|---|---|---|---|
| | | | 'Pirja' | | |
| B.396 | 81.0a | 506.1a | 57.1a | 6.3a | 0.71a |
| M.26 | 86.0a | 381.1b | 57.1a | 4.4b | 0.67a |
| $LSD_{0.05}$ | 14.9 | 49.3 | 8.5 | 1.4 | 0.16 |
| | | | 'Maikki' | | |
| B.396 | 102.0b | 603.4a | 70.0a | 5.9a | 0.69a |
| M.26 | 114.5a | 405.8b | 62.8b | 3.6b | 0.55b |
| $LSD_{0.05}$ | 3.2 | 16.4 | 6.3 | 0.3 | 0.08 |

Mean values followed by the same letter are not significantly different at $P \leq 0.05$.

### 5.2.2. Taste-related properties

The effect of rootstock (M.26, M.9 and B.396) on apple SSC, TA and SSC/TA concentration in summer cultivars ('Valge klaarõun', 'Maikki', 'Pirja', 'Krasnoje rannjeje') in 2003 and 2004 was studied. Rootstocks had a significant effect on apple taste-related properties of 'Pirja', 'Krasnoje rannjeje', 'Maikki and 'Valge klaarõun' in both years. However, the rootstock had the same effect in both years only on 'Valge klaarõun': trees on B.396 had significantly higher fruit TA concentration (**Paper II** Fig. 2). In 2003, 'Pirja', 'Krasnoje rannjeje' and 'Maikki' apples grown on B.396 had significantly lower TA concentration.

In 2003, fruit SSC ranged from 6 to 9 °Brix and in 2004, from 9 to 11 °Brix (**Paper II** Fig. 3). In 2003, rootstock had a significant effect only on 'Valge klaarõun': trees grown on M.26 had higher fruit SSC. In the second year, SSC was significantly lower in 'Maikki' and 'Valge klaarõun' apples grown on M.9. Rootstocks had no significant influence in either year on apple SSC in 'Krasnoje rannjeje' and 'Pirja'.

SSC and TA ratio of apples ranged from 5 to 30 being higher in 2004 than in 2003 (**Paper II** Fig. 4). In most of the cases, the influence of rootstock on apple SSC/TA was significant. Only in 2004, no significant influence in 'Maikki' was observed. In both years, SSC/TA was higher in fruits of 'Valge klaarõun' on M.26. In 2003, SSC/TA was lower in 'Valge klaarõun' on B.396 and in 'Pirja' on M.26. In 2004, the ratio was significantly higher in 'Krasnoje rannjeje' and 'Pirja' on M.9 and lower on M.26 and B.396, respectively.

### 5.2.3. Polyphenol composition

The effect of rootstocks B.396, M.26 and 'Antonovka' seedling on the concentration of polyphenols (CAT, CHL, PHL, QERT, QGAL and QTRI) in 'Talvenauding' apples in 2006 and 2007 was studied. In both years, the rootstocks significantly influenced the concentrations of different polyphenols in 'Talvenauding' apples (**Paper III** Table 2). Fruits from trees grown on vegetative rootstocks M.26 and B.396 had significantly higher concentration of polyphenols than those grown on 'Antonovka' seedlings. Significantly higher concentration of QGAL was measured in apples from trees grafted on B.396 in both years.

In 2006, significantly higher concentrations of CAT, PHL, QERT and QGAL were measured in apples from trees on vegetative rootstocks compared to 'Antonovka' seedling (**Paper III** Table 2). The concentrations of QERT (82 mg 100 $g^{-1}$ FW) and QGAL (46 mg 100 $g^{-1}$ FW) in the apples from trees grafted on B.396 were significantly higher than on M.26 where the concentration of same compounds were 72 and 31 mg 100 $g^{-1}$ FW, respectively. In 2007, B.396 had a positive effect on the concentration of CAT, QGAL and QTRI.

The area of a peak indicating the estimated content of total polyphenols ranged from 6781 to 15029 mAU min (data not shown). No significant differences were seen between the years and vegetative rootstocks. The estimated content of apple total polyphenols was significantly lower only in 'Talvenauding' grafted on 'Antonovka' seedlings.

## 5.3. Effect of the genotype on polyphenols

The concentration of 6 polyphenols (CAT, CHL, PHL, QERT, QGAL and QTRI) and the estimated content of total polyphenols in the peel of six apple cultivars ('Talvenauding', 'Krista', 'Liivi kuldrenett', 'Lobo', 'Cortland' and 'Antei') was measured. In the current study, PHL and QTRI were the most abundant polyphenols in the apple peel of studied cultivars (**Paper III**). Polyphenols occurred in the following decreasing order: PHL > QTRI> CAT > CHL > QGAL > QERT. Genotype had no significant effect on the order of polyphenols in the apple peel.

The effect of genotype on the content of polyphenols was significant, but differed between the years (**Paper III** Table 1). In 2006, 'Krista' had significantly higher concentration of phenolic compounds, except for CHL and PHL, compared to other cultivars. In 2007, 'Krista' had significantly lower concentrations of CAT, QERT and QGAL and no significant differences among other polyphenols compared to 2006. In 2006, 'Antei' had significantly lower concentration of CAT and 'Liivi kuldrenett' significantly lower content of QERT. However in 2007, the content of those polyphenols was markedly higher than in the other cultivars and in 2006.

The estimated content of total polyphenols varied significantly among the cultivars. In 2006, the total content of polyphenols was significantly higher in 'Krista', but in 2007 in 'Liivi kuldrenett' and 'Antei' (**Paper III** Fig. 3). No significant differences were seen among other cultivars. The mean effect of the cultivar showed significantly higher content of total polyphenols in 'Krista'.

# 6. DISCUSSION

## 6.1. The effect of Ca treatment

### 6.1.1. Mineral composition and storability (Paper I)

According to Sharples (1980), the apple fruit Ca content recommended for potential good keeping quality is 50 mg $kg^{-1}$ FW. In our study, only fruits from Ca-treated 'Talvenauding' in the second experimental year reached the value of 65 mg $kg^{-1}$ FW. The response was seen on apple postharvest quality, since spoilage of Ca-treated 'Talvenauding' was significantly lower compared to control after four months of storage. Based on these results, we may conclude that superficial scald disorder in 'Talvenauding' is related to mineral nutrition, which has not been stated before. Correlations between superficial scald and fruit mineral composition indicated that content of Ca and its ratios with K, N and Mg play an important role in severity of scald. Hence increasing fruit Ca by pre-harvest Ca sprays can improve postharvest quality of 'Talvenauding' apples.

Apple N content was also low in both years. The recommended level of N in apples is 500–700 mg $kg^{-1}$ FW (Sharples, 1980). In our experiment, $Ca(NO_3)_2$ solution was used twice and $CaCl_2$ once. Since N is more mobile in the plant, fruit N content increased significantly, but fruit Ca content did not increase during the first experimental year. Hence, despite the relatively low N level, the increase in N content without simultaneous increase in fruit Ca had a harmful impact on apple postharvest quality, probably by increasing respiration rate and ethylene production. The sufficient range for apple K content is said to be between 1300–1600 mg $kg^{-1}$ FW, fruit P above 110 mg $kg^{-1}$ FW and fruit Mg about 50 mg $kg^{-1}$ FW (Sharples, 1980). Thus, in the first experimental year, fruit K, P and Mg content was too low and in the second year, it was adequate. Despite that, in the second experimental year general fruit spoilage had a negative correlation with all of these nutrients, indicating that, for good keeping quality, content of these nutrients should be higher in studied cultivars.

Fruit N/Ca ratio was influenced mostly by cultivar: average value in 'Krameri tuviõun' fruits was higher (11.4 in both years) than in

‘Talvenauding’ fruits (5.7 in 2002 and 8.0 in 2003). Based on the results, ‘Krameri tuviõun’ could have better storage potential, because for good quality apples, the N/Ca should be either about 10 (Shear, 1974), or within the range 10–14 (Sharples, 1980; Dris, 1998). In our study, neither bitter pit nor physiological spot in ‘Krameri tuviõun’ showed a correlation with the N/Ca, which seems to confirm our previous statements, and indicates that these particular disorders are affected by nutrients other than Ca and N. Superficial scald in ‘Talvenauding’ correlated positively with N/Ca, indicating that the recommended N/Ca is too high for ‘Talvenauding’.

Bitter pit and physiological spot of ‘Krameri tuviõun’ correlated negatively with Mg and P content and Mg/Ca ratio in apples. Since bitter pit in ‘Krameri tuviõun’ correlated positively with Ca content, the benefit of Ca treatment in this cultivar is doubtful, as Ca and Mg are known to be antagonistic in fruit nutrition. Yuri *et al.* (2002) also found that sub-epidermal Ca levels at harvest explained only 53% of the variability in external bitter pit incidence after 120 days of storage. Retamales and Valdes (2001) stated that infiltration of Mg into fruit would provide a more reliable forecast. Lanauskas and Kvikliene (2006) also found that other nutrients, such as Mg, K, and N, may be involved in bitter pit development in apples and the ratios [K+Mg]/Ca and N/Ca can be used to predict fruit susceptibility. De Freitas *et al.* (2010) found higher concentrations of Mg and K and a higher ratio [K+Mg]/Ca in pitted fruit tissue. Even though numerous studies have confirmed that Ca treatment reduces bitter pit (Reid and Padfield, 1975; Neilsen and Neilsen, 2002), our results did not support these findings. Although, in the second year, the number of spoiled fruits of ‘Krameri tuviõun’ decreased, it was a result of the reduction of physiological spot, not bitter pit. It has been proposed that bitter pit is essentially the result of a gibberellin-induced increased susceptibility of the cell membranes to stress, and Ca only reduces the effect of gibberellins (Saure, 2002). Another explanation of bitter pit is through the importance of Ca in the cell wall structure. When the concentration of Ca in the apoplast is below the threshold required to maintain membrane structure and function, membrane leakiness results and pits develop in the tissue (de Freitas *et al.*, 2010). Competition between [K+Mg] and Ca for binding sites at the plasma membrane surface leads to more Ca being released to the apoplast solution. Consequently, less Ca will be bound to the plasma membrane, which will become leakier, eventually leading to plasmolysis, membrane breakdown, and cell death (de Freitas *et al.*, 2010).

### 6.1.2. Taste-related properties

Pre-harvest Ca sprays ($CaCl_2$) decreased fruit TA in 'Pirja' and 'Maikki' on M.26 and increased it in 'Maikki' on B.396. According to other researchers, the Ca treatment either increases (Raese and Drake, 1993; Dris and Niskanen, 1999; Wojcik, 2001) or has no influence (Casero *et al.*, 2010; Farag and Nagy, 2012) on TA in apples. The negative effect of Ca treatment on TA might have been a result of other influences: relationships with different mineral elements. Kadir (2004) found a negative correlation between fruit Ca concentration and TA.

Studies on different quality parameters on apples taste have shown TA to be the best predictor of apple acid taste and overall flavour (Harker *et al.* 2002). Differences of 0.08 g 100 $g^{-1}$ FW in TA between apples could evoke a response in perceived acid taste (Harker *et al.*, 2002) and a difference of 0.17 g 100 $g^{-1}$ FW a response in perceived sweet taste (Harker *et al.*, 2002). In our study, Ca treatment decreased TA in 'Pirja' and 'Maikki' on M.26 by 0.08 and 0.17 g 100 $g^{-1}$ FW respectively. The increase of TA caused by the Ca treatment in 'Maikki' on B.396 was less than 0.08 g 100 $g^{-1}$ FW. In the current study, based on instrumental measurements, we may suppose that Ca treatment affected apple acid and sweet taste when grown on M.26.

Although the Ca treatment had no effect on SSC, the treatment significantly increased SSC/TA in both cultivars grown on rootstock M.26. A similar effect of Ca treatment was noticed by Kadir (2004) with 'Jonathan' on EMLA 111. This author found SSC/TA to be positively related to flesh Ca and Mg and peel K content. Other researchers have found relationships between °Brix levels and TA (°Brix and TA ratio) and consumer acceptability (Vangdal, 1985; Harker *et al.*, 2002). SSC/TA is also a good predictor of apple flavour (Harker *et al.*, 2002). According to Kelt (1981), apples with SSC/TA between 15 and 20, have better taste properties. A ratio over 20 and below 15 significantly decreases the taste properties of apple. The treatment increased SSC/TA in 'Pirja' from 15.1 to 16.8 and in 'Maikki' from 14.9 to 17.1 when grown on M.26. In the current study, based on instrumental measurements, we may suppose that Ca treatment affected apple taste-related properties when grown on M.26. Klein *et al.* (1998) found that postharvest heated and/or Ca treated apples were perceived as crisper, sweeter and overall more acceptable than untreated fruits. However, sensory attributes of apples are not always adequately predicted by instrumental tests (Harker *et al.*, 2002). Also consumer preferences differ between countries (Cliff *et al.*, 2002).

### 6.1.3. Polyphenols and total antioxidant capacity (Paper IV)

The foliar Ca treatment was a beneficial tool to increase the polyphenol concentration in 'Maikki' on B.396. The treatment influenced apple mineral element composition so that it had a significant effect on fruit polyphenol concentrations. An increase in the Ca concentration had a positive influence on QERT, QGAL and QTRI, whereas an increase of N concentration caused a decrease of QERT. Previously, frequent negative correlations between N, Mg and N/Ca ratio and total flavonoids in the apple skin of standard 'Elstar' and 'Elshof' have been found by Awad and de Jager (2002). Also a positive correlation between fruit Ca and total flavonoids in the apple skin of standard 'Elstar' was found. A positive effect of Ca spray on apple phenolics has been observed by Sannomaru *et al.* (1998), who determined higher epicatechin, CHL and total polyphenol contents in Ca treated 'Starking' apples. In contrast, Unuk *et al.* (2006) concluded that the influence of applied N and crop load on apple polyphenol concentration was not consistent and no significant differences were found. Their results indicated that crop load or N fertilizers did not represent major regulatory factors in polyphenol formation in apples.

In our experiment, polyphenol concentrations in 'Pirja' were less affected by Ca treatment compared to 'Maikki'. Similarly with the latter cultivar, Ca treatment significantly increased the concentration of QTRI in fruits of trees on B.396. In 'Pirja', PHL and CYGL correlated with N and showed the tendency to increase or decrease respectively to the resulted increases or decreases of N concentration in apples. Awad and de Jager (2002) also found no positive correlation between Ca and total flavonoids in apples of 'Elshof' mutant. Based on our assumption that apple polyphenol concentration is influenced through the apple's mineral concentration, we can conclude that the modest effect of Ca treatment on polyphenols was because of its modest effect on the cultivar's mineral concentration.

Our findings clearly indicate that polyphenols have different antioxidant capacity. Although the quantity of QERT in 'Maikki' apples was negligible compared to several other polyphenols, it had an effect on apple TAC. Our results are in agreement with previously reported findings from Hagen *et al.* (2007), who found the strongest correlation between TAC and quercetin glycosides (quercetin-3-galactoside, quercetin-3-glucoside and quercetin-3-rhamnoside) in 'Aroma' apples. Lee *et al.* (2003) showed with six apple cultivars (Golden Delicious, Cortland, Monroe, Rhode Island Greening, Empire, NY674) that the estimated contribution of quercetin glycosides to TAC is the highest among major phytochemicals, whereas CHL and phloretin contribute minimally. In our experiment, PHL also provided a small though still significant contribution to TAC.

In the current experiment, a positive correlation between Ca and TAC in 'Maikki' apples was found. This was probably an indirect effect: Ca treatment affected apple fruit mineral composition, which had an effect on polyphenols and through them on TAC. The relationship between TAC and Ca became evident because the largest contributors to TAC (QERT, QGAL and QTRI) also had a positive correlation with Ca. Also, no negative relationship between TAC and N was found for the reason that QGAL and QTRI had no correlation with N. Hence, the effect of cultural practices on apple TAC is through the relationships between mineral concentration and polyphenols and polyphenols and TAC.

## 6.2. The effect of rootstock

### 6.2.1. Mineral composition

The Ca content in 'Maikki' apples was significantly higher on M.26 whereas in 'Pirja', there was no significant difference between the rootstocks. Compared to 'Pirja', 'Maikki' is a more vigorous cultivar and therefore the rootstocks had a significant effect on the mineral flow into the fruits. It can be supposed that the grafting site resistance with the semi-dwarfing rootstock M.26, compared to the dwarfing rootstock B.396, is smaller and the rootstock does not inhibit the flow of Ca into the fruits. Hence the higher content of Ca in fruits of 'Maikki' grown on M.26. In case of less vigorous cultivars, like ‘Pirja’, the grafting site resistance of M.26 and B.396 is weaker and has no influence on the flow of Ca from the roots to the fruits. Gąstoł and Poniedziałek (2005) found in ‘Jonica’ a greater amount of Ca in the wood above the grafting site and in the bark below the grafting site. They also found a higher content of Ca in ‘Jonica’ fruits grafted on M.9 compared to those grown on P 60. The fruit size of ‘Maikki’ on M.26 was significantly smaller than on B.396 (Karp and Noormets, unpublished data). Raese and Drake (2000) reported that unsprayed ‘Red Delicious’ fruits from M.7 rootstock had greater fruit peel Ca concentrations but were smaller than fruits from trees on M.26 rootstock.

The N content in ‘Pirja’ and ‘Maikki’ apples was affected by the rootstock being higher on B.396 in both cultivars. Although Jones (1971) and Sotiropoulos (2008) reported that exudates from dwarfing rootstocks contained lower N concentrations than those from more vigorous ones, the influence of rootstock on leaf N content can be more dependent on a growing site as reported by Rom *et al.* (1991). The growth vigour of the rootstock B.396 varies in different growing sites from semi-dwarfing to dwarfing (Haak, 2006). Although in Estonia, rootstock B.396 has been considered as dwarfing and M.26 as semi-dwarfing (Univer *et al.*, 2010), there are also data about B.396 being intermediate between dwarfing and semi-dwarfing in South Estonian conditions (Haak, 2006). In the current experiment, rootstock B.396 provided better N uptake in both cultivars. Previous analyses have shown higher SPAD values and therefore supposedly higher content of N in the leaves of ‘Maikki’ on B.396 compared to M.26 (Karp and Noormets, unpublished data). Studies have also shown that the content of macronutrients in the leaves depend on the rootstock (Poniedziałek *et al.*, 1993). Sotiropoulos (2008) found a higher concentration of N in the leaves of ‘Imperial Double Red Delicious’ grown on the seedling compared to the rootstocks M.7 and MM.106. Dris and Niskanen (1998) found a positive correlation between fruit and leaf N concentration.

### 6.2.2. Taste-related properties (Paper II)

In both years, significantly higher fruit TA was measured only in apples from ‘Valge klaarõun’ grown on B.396. In 2003, apples from ‘Pirja’, ‘Krasnoje rannjeje’ and ‘Maikki’ trees on B.396 had significantly lower

TA concentration. In contrast, Daugaard and Callesen (2002) found that the highest TA in 'Mutsu' fruits was on M.26, although considerable variations among years were also recorded. Skrzynski and Gastol (2006) also found that rootstock type and the year of study influenced TA.

TA has been shown to be the best predictor of acid taste and differences of 0.08 g 100 $g^{-1}$ FW in TA between apples can evoke a response in perceived acid taste (Harker *et al.*, 2002). A difference of 0.17 g 100 $g^{-1}$ FW in TA of apples can have a response in perceived sweet taste and a difference of 0.21 g 100 $g^{-1}$ FW in apple flavour (Harker *et al.*, 2002). In 2003, the difference in TA in 'Valge klaarõun' apples was 0.15 g 100 $g^{-1}$ FW and in 2004, it ranged from 0.07 to 0.09 g 100 $g^{-1}$ FW. In 2003, the difference in TA in 'Krasnoje rannjeje', 'Pirja' and 'Maikki' ranged from 0.23 to 0.24, from 0.06 to 0.07 and from 0.05 to 0.10 g 100 $g^{-1}$ FW, respectively. Harker *et al.* (2002) have determined the relationships between instrumental and sensory measurements and concluded that a difference of 0.21 g 100 $g^{-1}$ FW in TA can be perceived by consumers as a change in apple flavour. Based on instrumental measurements, in our study, rootstock B.396 might have an influence on apples acid taste and even on flavour of 'Krasnoje rannjeje' apples, since compared to apples from trees on other rootstocks the TA difference was up to 24 units.

In the first experimental year, the combination 'Valge klaarõun'/M.26 had significantly higher fruit SSC. In the second year, significantly lower SSC was measured in apples of 'Maikki' and 'Valge klaarõun' grown on M.9. In contrast to our results, earlier studies have found that fruits from cultivars grown on M.9 had higher SSC (Kviklys and Kvikliene, 2002; Skrzynski and Gastol, 2007; Kviklys *et al.*, 2012) and on M.26 lower SSC (Kviklys *et al.*, 2012). Rutkowski *et al.* (2005) stated that the influence of rootstock on SSC varied from year to year in 'Gala' fruits. Kviklys and Kvikliene (2002) found that fruits from trees on low-vigour rootstocks, such as P 22, M.9 or P 2, had much higher SSC than fruits from trees grown on more vigorous, M.26 or P 60 rootstocks. However, in another study with the same rootstocks, Kviklys *et al.* (2012) did not note a relationship between rootstock vigour and SSC. Controversially in our study, a tendency was noticed of low-vigorous rootstocks B.396 and M.9 causing lower SSC in vigorous and intermediate cultivars, such as 'Valge klaarõun', 'Maikki' and 'Krasnoje rannjeje', in 2003. In 2004, the same tendency was observed only in 'Valge klaarõun' and 'Maikki' on M.9. Hence, although the influence of rootstocks on apple SSC varies with year and depends on rootstock type (Daugaard and Callesen, 2002; Skrzynski and Gąstoł, 2006), it is also dependent on cultivar and growing site as concluded by Autio *et al.* (2001) and Kviklys *et al.* (2012).

Sweet taste is an important, but difficult attribute to predict using objective measurements (Harker *et al.*, 2002). °Brix has been shown to be the best and most convenient predictor of difference in taste when apple SSC differs by more than 1 °Brix (Harker *et al.*, 2002). In our experiment, rootstocks had a significant effect on SSC in apples, causing a difference of over 1 °Brix in concentration. Hence, based on the instrumental measurements we can assume that sweeter apples of 'Valge klaarõun' were obtained from trees grafted on M.26. In 2003, less sweet apples of 'Valge klaarõun' and 'Maikki' were obtained from trees on M.9.

SSC and TA are the most common parameters measured in chemical analyses related to fruit quality, and a change in the ratio between these parameters can have an impact on the taste of the apple. SSC/TA is a good predictor of apple flavour (Harker *et al.*, 2002). In our experiment, rootstocks had a significant effect on SSC/TA, but the effect differed with year, cultivar and rootstock. Only in 'Valge klaarõun' was a higher SSC/TA measured in both years when grown on M.26. Apples with SSC/TA between 15 and 20 have better taste-properties (Kelt, 1981). According to this, apples with better taste-properties were obtained only from 'Pirja' (on B.396 and M.9) in 2003, from 'Maikki' (on B.396 and M.26) in 2004, and from 'Valge klaarõun' on all rootstocks. Sensory attributes of apples are not always adequately predicted by instrumental tests (Harker et al., 2002). In Latvia, of early cultivars, consumers rated highest those with the highest SSC (16.96 °Brix) regardless of fruit size and high SSC/TA ratio (39.4) (Ikase and Seglina, 2008). Fruit firmness might be also one of the factors influencing evaluators. Harker *et al.* (2008) have suggested that firmness and SSC can explain an important portion of consumer preferences for apples. Taste, aroma and freshness were the three most important apple quality attributes taken into account by Swiss apple consumers (Péneau *et al.,* 2006). Estonian consumers preferred sour sweet apples (Moor *et al.*, 2013), as do Latvians (Ikase and Seglina, 2008). Latvians preferred cultivars with an acid content of 0.43 - 0.61 g 100 $g^{-1}$ FW (Ikase and Seglina, 2008). From local apples Estonians like 'Liivi kuldrenett' and 'Valge klaarõun', from imported apples 'Golden

Delicious' and 'Jonagold' (Moor *et al.*, 2013). As consumer preferences differ between countries (Cliff *et al.*, 2002), the acceptable SSC/TA by consumers can also differ. Because Estonian apples tend to have higher TA than imported apples, the suggested range of SSC/TA from 15 to 20 by Kelt (1981) is acceptable to Estonian consumers, but might not be acceptable by those of other nationalities.

#### 6.2.3. Polyphenols (Paper III)

Apples from 'Talvenauding' trees grown on vegetative rootstocks M.26 and B.396 had significantly higher concentration of polyphenols than those grown on 'Antonovka' seedlings. Previous research conducted in Estonia demonstrated that dwarfing vegetative rootstocks successfully inhibit the growth of vigorous cultivars (Haak, 2003; Univer *et al.*, 2013). Since 'Talvenauding' is a vigorous cultivar, the dwarfing effect of B.396 and M.26 might have had a beneficial influence on the concentrations of polyphenols, because canopies with weaker shoot growth provide better light conditions for fruits. Fruits from dwarf and high yielding trees showed the highest percentage of fruit surface covered by red colour (Kviklys *et al.,* 2012). A tendency to poorer coloration was noted with increasing rootstock vigour (Kviklys *et al.*, 2012). According to Hagen *et al.* (2007), the concentration of flavonoids and the level of total phenols are higher in the peel of sun-exposed apples compared to shade-grown apples.

### 6.3. Apple polyphenol concentration as affected by genotype (Paper III)

In the current study, with 'Talvenauding', 'Krista', 'Liivi kuldrenett', 'Lobo', 'Cortland' and 'Antei', PHL and QTRI were the most abundant polyphenols in the apple peel. Polyphenols occurred in the following decreasing order: PHL > QTRI> CAT > CHL > QGAL > QERT. Genotype had no significant effect on the order of polyphenols in the apple peel. These results are similar to those found by Lu and Foo (1997). D'Abrosca *et al.* (2007) also found that apple peel was rich in PHL, phloretin-2'-xyloglucoside, CAT and epicatechin.

The experiment demonstrated a large difference in the concentration of polyphenols in different apple cultivars showing the significant influence of genotype. The largest variation of concentrations between the cultivars occurred in QERT and QGAL. In 2006, ‘Krista’ and ‘Lobo’ had a 23-fold difference in QERT concentration. Earlier experiments with 19 apple cultivars in Poland have shown a 9-fold difference in CHL (Łata et al., 2009) and an experiment with three apple cultivars in Japan reported a 15-fold difference in CAT content (Kondo et al., 2002). Researchers have indicated the beneficial effect of QERT on human health (Knekt et al., 2002; Ansari et al., 2009). The current study showed a significant difference in QERT concentration among cultivars and years. In 2006, the concentration of QERT and QTRI was significantly higher in ‘Krista’ and in 2007, in ‘Liivi kuldrenett’. The cultivar experiment indicated that the apple peel with higher content of total polyphenols had also higher content of flavonols and CAT. As summarized by Wojdyło et al. (2008), genotypes with the highest phenolic concentration had simultaneously high content of flavonols.

Yearly differences in weather conditions also had an impact: a significantly higher concentration of phenolic compounds was found in 2007. We can assume that the increased content of polyphenols in 2007 was caused by longer sunlight hours, less precipitation and higher temperature in August 2007 compared to August 2006. Genetic background, developmental stage and environmental factors, such as nutrient availability, temperature and particularly light influence the synthesis of polyphenols (Saure, 1990). Sunlight induces many enzymes involved in flavonoid synthesis (Treutter, 2001). In 2007, all the apples had a better red skin colour and higher polyphenol concentration (except QERT). Even the yellow apples of ‘Liivi kuldrenett’ had a red blush, which might have been due to the 68% more sunlight hours in August 2007 compared to the same period in 2006. Better light conditions before harvesting probably caused the higher concentration of polyphenols in the apple peel in 2007. These results are in agreement with Hagen et al. (2007) who found that the red to green colour values of the apple skin correlated very well with the peel content of phenolic compounds and ascorbic acid in ‘Aroma’ apples. They also implied that the colour of the apple skin might provide useful information about the health value of the apples.

# 7. CONCLUSIONS

**From pre-harvest Ca treatment experiments it can be concluded:**

- The Ca treatment ($CaCl_2$ and $Ca(NO_3)_2$) did not reduce bitter pit, but reduced other physiological disorders: physiological spot and superficial scald.

- By combining pre-harvest Ca treatment with certain rootstocks, it is possible to manipulate apple taste related properties; primarily to increase soluble solids and titratable acidity ratio in apples.

- The metabolism of phenolic compounds can be influenced via mineral nutrition, which is affected by different agricultural practices. More attention should be paid to the combined effects, which might be different from the influence each factor has separately; for instance, the influence of the Ca treatment depended on rootstock.

- By influencing apple mineral composition with Ca treatment, it is possible to increase the polyphenol concentration and finally, also the TAC of apples. Different polyphenols were differently affected by the fruit mineral composition. Largest contributors to TAC, such as QERT, QTRI and QGAL. Rootstock and regulation of N and Ca concentrations in apples caused almost a doubling of TAC in fruits.

**From rootstock experiments it can be concluded:**

- Rootstocks affected mineral composition of apples and through that also the concentration of certain polyphenols. Compared to the seedling, the vegetative rootstocks increased significantly the polyphenol concentration of apples: higher concentrations of CAT, PHL, QERT and QGAL were measured

- The influence of rootstocks on apple taste-related properties was significant, but varied with year and cultivar. A consistent effect appeared in 'Valge klaarõun'.

**Genotype experiments demonstrated:**

- In all studied cultivars, polyphenols occurred in the following decreasing order: PHL > QTRI> CAT > CHL > QGAL > QERT.

- A large difference in the concentration of polyphenols between 'Talvenauding', 'Krista', 'Liivi kuldrenett', 'Lobo', 'Cortland' and 'Antei' was found. The largest variation of concentrations occurred in QERT and QGAL. 'Krista' and 'Lobo' had a 23-fold difference in QERT concentration.

**Practical use of the results**

The results of the current thesis are usable by apple growers, food processers and consumers. Based on our results it is possible for apple growers to reduce apple storage losses and complement cultivar selection by including cultivars prone to Ca deficiency but otherwise with valuable quality properties. Depending on a cultivar and rootstock, spraying with Ca fertilizers is not always necessary. By choosing certain cultivar/rootstock combinations, the grower can reduce production costs with respect to expense of Ca fertilizers. With different agricultural practices it is possible to increase apple health-related properties (content of polyphenols, TAC) and through that give the product an additional value and increase the grower's competitiveness in the market.

To food processers (apple juice, puree, baby food producers etc.) the experiment's results give information about the biochemical composition of local apple cultivars, which enables them to make more knowledgeable choices of raw materials. Consequently, it is possible to produce food with higher health-beneficial properties or with better taste and appearance. For example, in purees and apple cakes, fruits with high TA are preferred. In addition to health benefits, concentration of certain polyphenols influences significantly apple browning when sliced and in contact with air, which in turn influences the colour of apple products.

To increase consumer awareness of food composition, it would be valuable to add information about the biochemical composition of different apple cultivars to food databases. In the current study, 23-fold differences between polyphenol concentrations of different cultivars were found. Hence, to give information as “average apple composition” is not justified.

**Future research objectives would be to determine:**

- the polyphenol composition in yet not studied apple cultivars recommended for commercial production in Estonia and other Baltic states in order to find cultivars suitable for fresh consumption and, for example, cider production;
- combined effect of different agricultural practices on the concentration of polyphenols with higher antioxidant capacity;
- combined effect of different agricultural practices on apple taste-related properties.

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# SUMMARY IN ESTONIAN

## MUUTUSED ÕUNTE BIOKEEMILISES KOOSTISES SÕLTUVALT AED-ÕUNAPUU (*Malus domestica* Borkh.) POOKEALUSEST JA KALTSIUMIGA VÄETAMISEST

### Sissejuhatus

Baltimaades on õuntega seotud teadustöö peamiseks eesmärgiks olnud talvekindlate pookealuste ja sortide väljaselgitamine. Probleemideks on lühike vegetatsiooniperiood, talvised madalad õhu- ja mullatemperatuurid ning varakevadised ulatuslikud ööpäevased temperatuurikõikumised. Saagi kvaliteedi uurimustes on tähelepanu pööratud õuna suurusele, välimusele ja säilivusele. Erinevates *in vitro* ja *in vivo* uuringutes on selgunud, et puu- ja köögiviljades sisalduvatel bioaktiivsetel ainetel nagu karotenoidid, flavonoidid, isoflavonoidid ja fenoolsed happed, on oluline mõju inimese tervisele. Epidemioloogilistes uurimustes on täheldatud, et õunte tarbimine vähendab riski haigestuda vähki, südameveresoonkonna haigustesse, astmasse ja II tüübi diabeeti. Seetõttu on hakatud teadusuuringutes rohkem tähelepanu pöörama õunte biokeemilisele koostisele, eriti polüfenoolide sisaldusele ja antioksüdatiivsele aktiivsusele. Samas on endiselt olulised ka õunte välimus, maitse ja säilivus.

Õuntes sisalduvad mitmed bioaktiivsed ained, kuid kõrge antioksüdatiivse aktiivsuse põhjuseks peetakse eelkõige mitmeid polüfenoole. Peamised õuntes leiduvad polüfenoolid on hüdroksükaneelhappe derivaadid (klorogeenhape), flavonoolid (kvertsetiini glükosiidid), flavanoolid (katehhiin, epikatehhiin, protsüanidiin) ja dihüdrohalkoonid (floridisiin). Polüfenoolide sisaldus on sorditi ja samuti vilja erinevates osades erinev: võrreldes viljalihaga on koores ja selle all asuvas kihis erinevus 1,2–3,3 kordne. Mitmed polüfenoolid nagu kvertsetiin ja selle glükosiidid ning antotsüaanid, sisalduvad ainult õuna koores. Samas epikatehhiini, protsüanidiine, floridisiini ja klorogeenhapet on leitud ka viljalihast. Erinevad polüfenoolid on erineva antioksüdatiivse aktiivsusega. Katsetulemustest on selgunud, et suurema antioksüdatiivse võimega on kvertsetiin ja selle glükosiidid. On leitud, et koor on 1,5–9,2 korda suurema antioksüdatiivse aktiivsusega kui viljaliha ja seetõttu on oluline tarbida õuna koos koorega.

Lisaks sordiomadustele mõjutavad polüfenoolide sisaldust õuntes istandiku geograafiline asukoht, vegetatsiooniperioodi ilmastik, agrotehnika (väetamine, taimekaitse, võralõikus), puu saagikus, vilja asukoht võras ja vilja küpsusaste. Pookealuse mõju õunte polüfenoolide sisaldusele on vähe uuritud. Samas on rohkelt andmeid pookealuse mõju kohta vilja suurusele, välimusele, puu kasvule ja toiteelementide omastamisele ning seetõttu võis eeldada ka pookealuse mõju õunte polüfenoolide sisaldusele. Peamistes õunakasvatuspiirkondades levinud õunasortide kohta on olemas andmed viljades sisalduvate erinevate biokeemiliste ühendite, kaasaarvatud polüfenoolide kohta. Eestis kasvatatavate õunasortide polüfenoolide sisalduse kohta puudusid enne käesolevat uurimistööd teaduskirjanduses andmed.

Õunakasvatuses on lisaks põhiväetamisele (juure kaudu väetamine) levinud ka juureväline ehk lehe kaudu väetamine, mille käigus pritsitakse väetiselahusega õunapuu võra. Eelkõige kasutatakse juurevälist väetamist kaltsiumipuudusest tingitud füsioloogiliste häirete vähendamiseks viljadel. Samas ei ole ühtset arvamust pritsimissageduste ja -aegade ning kaltsiumväetiste normide kohta. Teistes maades antud soovitused ei pruugi meie tingimustesse sobida, sest väetamise mõju sõltub sordist ja kasvukoha tingimustest. Seetõttu oli vajalik välja selgitada kaltsiumipreparaatide mõju kohalikele probleemse säilivusega sortidele.

Käesoleva doktoritöö eesmärgiks oli välja selgitada:

- õunapuude kaltsiumilahusega pritsimise mõju õunte mineraalelementide ja füsioloogilistele häiretele;
- kaltsiumlahusega pritsimise mõju erinevatel alustel puude õunte maitsega seotud omadustele, polüfenoolide sisaldusele ja antioksüdatiivsele aktiivsusele;
- pookealuste mõju õunte mineraalelementide, maitsega seotud omadustele ja polüfenoolide sisaldusele;
- genotüübi mõju õunte polüfenoolide sisaldusele.

**Materjal ja metoodika**

Käesoleva doktoritöö raames toimunud katsed viidi läbi kahes Lõuna-Eestis paiknevas õunaistanduses: Eesti Maaülikooli Rõhu Katsejaamas ja tootmisühistus Vasula Aed (tabel 3).

**Tabel 3.** Ülevaade käesoleva doktoritöö raames toimunud katsetest

| Mõjufaktor | Katseobjekt | Analüüsitud parameetrid | Aasta | Istanduse asukoht |
|---|---|---|---|---|
| Juurevälised Ca-väetised $CaCl_2$ ja $Ca(NO_3)_2$ | 'Krameri tuviõun', 'Talvenauding', alus: 'Antonovka' seemik | Ca-, N-, P-, K-, Mg-sisaldus, koore pruunistumine, kaltsiumipuudus, kooretäpilisus | 2002-2004 | TÜ Vasula Aed |
| Juureväline Ca-väetis $CaCl_2$ | 'Pirja', 'Maikki', alused: M.26, B.396 | Ca- ja N- sisaldus, orgaanilised happed, mahla kuivaine ja eelnevate suhtarv, CAT, CHL, PHL, QERT, QGAL, QTRI, CYGL, TAC | 2008 | Rõhu Katsejaam |
| Pookealused M.26, M.9, B.396 | 'Valge klaarõun', 'Maikki', 'Pirja', 'Krasnoje rannjeje' | Orgaanilised happed, mahla kuivaine ja eelnevate suhtarv | 2003 2004 | |
| Pookealused M.26, B.396, 'Antonovka' seemik | 'Talvenauding' | CAT, CHL, PHL, QERT, QGAL, QTRI | 2006 2007 | |
| Genotüüp | 'Talvenauding', 'Krista', 'Liivi kuldrenett', 'Lobo', 'Cortland', 'Antei' , alus: 'Antonovka' seemik | CAT, CHL, PHL, QERT, QGAL, QTRI | 2006 2007 | |

CAT – katehhiin, CHL – klorogeenhape, PHL – floridsiin, QERT – kvertsetiin, QGAL – kvertsetiini galaktosiid, QTRI – kvertsetriin, CYGL – tsüanidiini glükosiid.

Rõhul olid seemikalusel sügis- ja talisordid istutatud 1986. a, suvisordid ja nõrgakasvulistele alustele poogitud sordi 'Talvenauding' (punane kloon) puud 2001.a. TÜ Vasula Aed tootmiskatses olid puud istutatud 1975. a.

Säilituskatses eraldati igakuiselt riknenud õunad tervetest. Riknenud õuntel määrati sorditi erinevate füsioloogiliste häirete esinemine (**artikkel I**). Õunte N-sisaldus määrati Kjeldahli meetodil, P- ja Mg-sisaldus FIA-meetodil, K-sisaldus leekfotomeetriliselt ja Ca- sisaldus ICP spektromeetriga (**artiklid I** ja **IV**). Orgaaniliste hapete sisaldus määrati 5 g uhmerdatud proovist valmistatud vesilahust 0,1 N NaOH lahusega tiitrides (**artikkel II**). Mahla kuivaine määrati refraktomeetriga (**artikkel II**). Polüfenoolide sisalduse määramiseks ja identifitseerimiseks kasutati kõrgefektiivset vedelikkromatograafi (HPLC) koos tandem mass-spektromeetriga (MS/MS) (**artiklid III** ja **IV**). Polüfenoolide üldine hinnatav sisaldus arvutati kromatograafiliste kõverate pindalade (AUC) summana lainepikkusel 280 nm (**artikkel III**). Õunte antioksüdatiivne aktiivsus määrati etanooliekstraktis DPPH (2,2'-difenüül-1-pikrüülhüdrasüül) – meetodil (**artikkel IV**).

**Tulemused**

Õunapuude pritsimine kaltsiumväetiste ($CaCl_2$ ja $Ca(NO_3)_2$) lahustega mõjutas õunte mineraalset koostist, kuid mõju oli sorditi erinev (artikkel I). Esimesel katseaastal vähendas väetamine sordil 'Krameri tuviõun' oluliselt Ca-sisaldust ja suurendas N-, K- ja P-sisaldust. Sordil 'Talvenauding' suurendas väetamine õunte K- ja vähendas P-sisaldust. Teisel aastal oli väetamise mõju sordile 'Krameri tuviõun' vastupidine: Ca-sisaldus suurenes ja N- ja P-sisaldused vähenesid. Sordil 'Talvenauding' suurendas väetamine oluliselt Ca-sisaldust ja vähendas Mg-sisaldust.

Kaltsiumiga väetamise mõju õunte mineraalsele koostisele ja selle kaudu füsioloogilistele häiretele sõltus kasvuperioodi ilmastikust (**artikkel I**). Esimesel katseaastal ei vähendanud väetamine füsioloogiliste häirete esinemist ega vähendanud seeläbi ka säilituskadu. Teisel aastal vähendas väetamine säilituskadusid kuni neljanda säilituskuuni: sordil 'Krameri tuviõun' vähenes kooretäpilisuse esinemine ja sordil 'Talvenauding' koore pruunistumine. Sordi 'Krameri tuviõun' puhul korreleerusid nii kaltsiumipuudus kui ka kooretäpilisus negatiivselt Mg- ja P- sisaldusega ning Mg/Ca suhtega. Koore pruunistumine sordil 'Talvenauding' korreleerus negatiivselt Ca-sisaldusega ja positiivselt K/Ca, N/Ca ja Mg/Ca suhetega.

Väetamine ei mõjutanud 'Maikki' ja 'Pirja' õunte mahla kuivaine sisaldust (avaldamata andmed). Pookealusel M.26 vähendas kaltsiumväetis oluliselt orgaaniliste hapete sisaldust mõlema sordi puhul. Samas alusele B.396 vääristatud sordil 'Maikki' suurendas väetamine oluliselt orgaaniliste hapete sisaldust. Mahla kuivaine ja orgaaniliste hapete suhet suurendas väetamine mõlemal sordil ainult alusel M.26.

Juurevälise kaltsiumiga ($CaCl_2$) väetamise mõju polüfenoolide sisaldusele sõltus pookealusest (**artikkel IV**). Alusel B.396 suurendas väetamine kvertsitriini sisaldust, kuid alusel M.26 oli mõju vastupidine – kvertsitriini sisaldus vähenes. 'Maikki' õuntes mõjutas polüfenoolide sisaldust mineraalelementide sisaldus: kvertsitriinil, kvertsetiini galaktosiidil ja kvertsetiinil oli positiivne seos Ca- ja negatiivne seos N-sisaldusega. Kvertsitriin, kvertsetiini galaktosiid ja kvertsetiin mõjutasid omakorda oluliselt antioksüdatiivset aktiivsust.

Pookealustel M.26 ja B.396 oli oluline mõju sortide 'Pirja' ja 'Maikki' õunte mineraalsele koostisele (avaldamata andmed). Sordil 'Pirja' avaldas alus olulist mõju N- sisaldusele ja N/Ca suhtele: variandi B.396 puhul olid vastavad näitajad suuremad. Alusel B.396 kasvanud sordi 'Maikki' õuntes oli oluliselt suurem N- ja Mg- sisaldus ning N/Ca ja Mg/Ca suhe. Ainult Ca- sisaldus oli oluliselt suurem alusele M.26 vääristatud puude õuntes.

Pookealustel oli oluline mõju suveõunte maitset mõjutavatele parameetritele (**artikkel II**). Pookekombinatsiooni 'Valge klaarõun'/B.396 viljad olid oluliselt suurema orgaaniliste hapete sisaldusega mõlemal aastal. Pookealuse mõju mahla kuivaine sisaldusele ilmnes 2003. a vaid sordil 'Valge klaarõun' – positiivne mõju oli alusel M.26. Sama tendentsi võis märgata ka teisel aastal. 2004. a oli M.9 kasvanud sortide 'Valge klaarõun' ja 'Maikki' õunad oluliselt väiksema mahla kuivaine sisaldusega. Õunte mahla kuivaine ja orgaaniliste hapete suhe jäi vahemikku 5-30. Alusele M.26 vääristatud sordil 'Valge klaarõun' oli mahla kuivaine ja orgaaniliste hapete suhe oluliselt suurem mõlemal aastal.

Pookealused avaldasid olulist mõju 'Talvenaudingu' õunte polüfenoolide sisaldusele (**artikkel III**). Võrreldes 'Antonovka' ja M.26-ga, oli B.396 variandi õuntes floridisiini, kvertsetiini ja kvertsetiini galaktosiidi sisaldus mõlemal aastal oluliselt suurem. Ainult klorogeenhappe sisalduse puhul ei olnud seemikaluse ja vegetatiivaluste vahel olulist erinevust.

Vegetatiivaluste katses suvesortidega selgus, et võrreldes alusega B.396, oli alusele M.26 vääristatud sordil 'Maikki' enamike polüfenoolide sisaldus suurem, kuid sordil 'Pirja' suurenes ainult floridisiini sisaldus (**artikkel IV**).

Sordivõrdluskatses kuue õunasordi ('Talvenauding', 'Krista', 'Liivi kuldrenett', 'Lobo', 'Cortland', 'Antei') analüüsimisel selgus, et polüfenoolid esinesid õuna koores järgmises kahanevas järjekorras: floridisiin > kvertsitriin > katehhiin > klorogeenhape > kvertsetiini galaktosiid > kvertsetiin (**artikkel III**). Suurima katehhiini ja flavonoolide sisaldustega sortidel oli ka suurim polüfenoolide üldsisaldus. Olulised erinevused sortide vahel esinesid kvertsetiini ja kvertsetiini galaktosiidi sisaldustes: suurima sisaldusega olid sortide 'Krista' ja 'Liivi kuldrenett' õunad. Kvertsetiini sisaldus oli 'Krista' õuntes 23 korda suurem kui 'Lobo' õuntes (vastavalt 117,6 mg 100 $g^{-1}$ VM ja 5,8 mg 100 $g^{-1}$ VM).

## Järeldused

Läbi viidud katsest selgus esmakordselt, et sordi 'Talvenauding' õuntel vähendas juureväline kaltsiumiga väetamine seni kaltsiumipuudusega mitte seostatud füsioloogilist häiret – koore pruunistumist. 'Krameri tuviõuna' kaltsiumipuuduse vältimisel on oluline tagada mitte niivõrd kõrgem Ca- sisaldus, vaid optimaalne Mg/Ca suhe õunas.

Juurevälist kaltsiumiga pritsimist teatud alustega kombineerides, on võimalik mõjutada õunte maitseomadusi. Pritsimine vähendas orgaaniliste hapete sisaldust ja suurendas mahla kuivaine ja orgaaniliste hapete suhet õuntes.

Pookealused mõjutasid õunte mineraalset koostist: poolnõrgakasvulisele alusele poogitud puude õuntes oli kõrgem Ca- sisaldus ja nõrgakasvulisel alusel N- sisaldus.

Pookealustel oli oluline mõju suveõunte maitset mõjutavatele parameetritele kuid see varieerus aastati ja sorditi. Aastati sarnane mõju oli sordil 'Valge klaarõun': pookealuse B.396 mõju maitset mõjutavatele parameetritele oli piisav, et olla tuntav muutusena õuna maitses.

Polüfenoolide sisaldus oli Eestis kasvatatavates õuntes väga varieeruv. Polüfenoolidel on erinev antioksüdatiivne aktiivsus; inimtervise seisukohalt on oluline suurendada eelkõige kõrge antioksüdatiivse aktiivsusega polüfenoolide sisaldust. Selgus, et fenoolsete ühendite metabolismi saab suunata erinevate agrotehniliste võtetega, seejuures on oluline arvestada erinevate tegurite (näiteks juurevälise väetamise ja pookealuse) koosmõju. Mõjutades agrotehniliste võtetega õunte mineraalset koostist, on võimalik suurendada polüfenoolide sisaldust ja seega ka õunte antioksüdatiivset aktiivsust. Erinevad polüfenoolid olid õuna mineraalse koostise kaudu erinevalt mõjutatavad. Suurima antioksüdatiivse aktiivsusega polüfenoolidel (kvertsitriin, kvertsetiini galaktosiid, kvertsetiin) oli positiivne seos Ca- ja negatiivne seos N-sisaldusega õunas. Pookealuse valiku ja kaltsiumi- ning lämmastikusisalduse reguleerimise kaudu õnnestus käesolevas uurimustöös suurendada õunte antioksüdatiivset aktiivsust isegi kaks korda.

## Töö tulemuste rakendamise võimalused praktikas

Doktoritöö tulemused on rakendatavad nii õunakasvatajate-, töötlejate kui ka tarbijate poolt. Tootjal on võimalik uurimistööst saadava info põhjal vähendada õunte säilituskadusid ja täiendada sordivalikut kaltsiumipuudusele vastuvõtlike, kuid muude omaduste poolest väärtuslike sortidega. Sõltuvalt pookealusest ja sordist ei ole Ca-väetise andmine alati otstarbekas, seega on tootjal võimalik teatud sordi- ja pookekombinatsioonide kasutamise korral Ca- väetise arvelt tootmiskulu vähendada. Suurendades erinevate agrotehniliste võtetega õunte tervislikkusega seotud parameetreid (polüfenoolide sisaldust, antioksüdatiivset aktiivsust), annab see tootele lisaväärtust ja suurendab tootja konkurentsivõimet turul.

Toidu töötlejatele (õunamahlade-, püreede-, lastetoitude jne. valmistajaile) annavad katsetulemused infot kohalike õunasortide biokeemilise koostise kohta, võimaldades teadlikumalt valida toorainet ja valmistada kvaliteetsemaid tooteid. Lisaks inimtervisele olulistele aspektidele, on väga olulised ka toodete välimust ja maitset mõjutavad karakteristikud. Püreedes on näiteks eelistatumad kõrge orgaaniliste hapete sisaldusega õunad, polüfenoolide sisaldus mõjutab väga oluliselt õuna pruunistumist õhuga kokkupuutel ning seetõttu õunatoodete värvust.

Tarbija teadlikkuse tõstmiseks oleks oluline toidu koostise andmebaasidesse lisada info erinevate õunasortide koostise kohta. Antud töö tulemustest selgus, et inimtervisele oluliste polüfenoolide sisalduses võib õunasortide vahel olla isegi kuni 23-kordne erinevus, seega ei ole õigustatud koostise esitamine „keskmise õuna“ kohta.

**Edasist uurimist vajavad küsimused:**

- Eestis äriaedades kasvatamiseks soovitatavate ja seni uurimata sortide õunte polüfenoolide sisaldus, selgitamaks värskelt tarbimiseks ja näiteks siidriõunteks sobivaid sorte;
- erinevate agrotehniliste võtete koosmõju kõrge antioksüdatiivse aktiivsusega polüfenoolide sisaldusele õuntes;
- erinevate agrotehniliste võtete koosmõju õunte maitseomadustele.

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# I

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**ORIGINAL ARTICLE**

# Post-harvest disorders and mineral composition of apple fruits as affected by pre-harvest calcium treatments

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**Abstract**
The effect of pre-harvest calcium sprays, $CaCl_2$ and Ca $(NO_3)_2$, on apple (*Malus domestica*) N, P, K, Ca and Mg content, storability and incidence of physiological disorders (superficial scald, bitter pit, physiological spot) was studied using two cultivars, 'Krameri Tuviõun' and 'Talvenauding'. In the first year calcium treatment did not reduce any physiological disorders or loss of marketable yield. In the second year calcium treatment reduced storage losses until four months after storage. Bitter pit in 'Krameri Tuviõun' was unaffected by calcium treatment, but physiological spot of 'Krameri Tuviõun' and superficial scald of 'Talvenauding' were reduced by calcium treatment in the second year. Both physiological disorders of 'Krameri Tuviõun' correlated negatively with Mg and P content and Mg/Ca ratio in apples. Superficial scald of 'Talvenauding' correlated negatively with Ca content and positively with K/Ca ratio, N/Ca ratio and Mg/Ca ratio in fruits. The conclusion can be made that content of Ca and its ratios with other nutrients plays an important role in the development of superficial scald on 'Talvenauding' fruits, and increasing Ca content in fruit can therefore be recommended for improving post-harvest quality of this cultivar. Since bitter pit in 'Krameri Tuviõun' was not reduced by calcium treatment, it would be worth trying other measures for this cultivar.

**Keywords:** *Bitter pit, physiological spot, quality, storage, superficial scald.*

## Introduction

Common problems for apple producers worldwide are physiological disorders which lead to remarkable loss of marketable yield during storage. Numerous studies have been carried out to explain the causes and find cures for these apple disorders. Most of these studies were conducted in typical apple-growing regions, and only little is known concerning apple disorders in Nordic countries. Dris and Niskanen (1998), who studied nutritional status of apple orchards in Finland, found that Nordic countries have to follow the recommendations from other countries, since there are few data available about the Nordic regions.

In many parts of the world, calcium sprays are used as a routine protective measure, in order to prevent or reduce bitter bit of apples (Saure, 2002). However, the threshold values of calcium below which bitter pit is likely to occur, or above which fruit may be expected to be safe, vary from year to year and from district to district, for still unknown reasons. There are different opinions on treatment time and quantities. Several scientists suggest that calcium should be applied to the apple trees in the second part of the growing season and treatment should be carried out four to six times (Benavides et al., 2001). At the same time other researchers recommend that calcium be applied to apple trees more than six times during growth (Kadir, 2004). Thus, none of the calcium-based prediction schemes that have been developed, partially incorporating many additional variables, is generally applicable or sufficiently reliable under varying conditions (Saure, 1996).

Our first hypothesis was that apples grown in Nordic countries could have different responses to pre-harvest calcium treatment from those of apples grown in southern regions. This hypothesis is based on numerous statements in the literature indicating that the effect of calcium treatment varies among

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seasons (Saure, 2002; Saure, 1996; Yuri, 1995), which suggests that it might vary even more between different climatic regions. Secondly, we hypothesized that mineral nutrition of apples could also affect other physiological disorders besides bitter pit.

The aim of the present research was to study the influence of pre-harvest calcium treatment on mineral composition and post-harvest quality of apples in the Nordic region and to find relationships between different physiological disorders and apple nutrients.

## Materials and methods

### *Plant material and pre-harvest treatments*

The experiment began in June 2002 and finished in April 2004. The following apple cultivars were used for the experiment: 'Krameri Tuviõun': fruit weight 80–110 g, ripens in November and is meant for storage until January. 'Talvenauding': fruit weight 80–125 g, ripens in November and is meant for storage until March. 'Antonovka' was used as seedling rootstock for all cultivars.

Apple trees were grown in a commercial orchard of the company Vasula Aed, which is situated in Tartu County, south Estonia (58°28′N and 26°44′E). The plantation was 27 years old; rejuvenation was carried out in 1997, and routine thinning has been carried out every year. Apple trees were planted with a distance of about 8 m between rows and 4 m within the row. No irrigation system was used in the plantation.

The soil in the experimental area is a sandy loam, endoeutri-haplic luvisol. The depth of the humus horizon ranges from 24 to 30 cm. Soil samples were taken in September using a steel auger at depths of 20–50 cm. The samples comprised a mixture of 10 subsamples from both the control trees (unfertilized) and calcium-treated trees areas. The following elements were determined from soil samples: P- (ammonium lactate extractable), K- (ammonium lactate extractable), Ca- and Mg- (1 *M* ammonium acetate extract, pH 7.0). Soil analyses indicated that there was no deficiency of any nutrients in the soil (Table I).

Foliar fertilization of apple trees was applied as follows: in 2002 $Ca(NO_3)_2$ 1% solution was applied on 9 and 30 July at a rate of 660 l ha$^{-1}$, and $CaCl_2$ 0.5% solution was applied on 22 August at the same rate. In 2003 $Ca(NO_3)_2$ was applied on 14 and 28 July and $CaCl_2$ on 8 August and 1 September.

Apples were harvested during the second and third week of September in 2002 and 2003, respectively. Samples of 300 fruits per plot (50 fruits in six replications) were collected at random. First quality apples were picked according to an equatorial pattern (North-South-East-West) from the outside of the trees avoiding fruit situated at the top, the bottom and also deep inside the canopy. Apple fruits were stored in a commercial cool store of the company Vasula Aed at 2–5°C and 80–85% RH. Fifty fruits were placed on one layer in air-permeable plastic boxes. The storage period for 'Krameri Tuviõun' was four months and for 'Talvenauding' six months.

### *Measurements and analyses*

Spoiled apples were removed from healthy apples monthly and divided into categories, which were 'Talvenauding' fruits with superficial scald and other quality loss symptoms; and 'Krameri Tuviõun' fruits with calcium deficiency symptoms (bitter pit), physiological spot, and others. The fruit content of Ca, N, P, K and Mg was determined after harvest. N concentration of air-dried samples was determined by the Kjeldahl method. The method involves digestion of the sample in sulphuric acid using the Kjeldahl Cu catalyst to convert the protein nitrogen to ammonium sulphate. Ammonia is then liberated by alkaline distillation using the automatic analyser Kjeltec Auto 1030. P and Mg concentrations were measured from a Kjeldahl digest using the flow injection analyser 'FIAstar 5000'; K concentration was determined flamephotometrically by an air-acetylene flame. P was determined at a wavelength of 720 nm by the stannous chloride method. Mg was determined by the Titan Yellow method at a wavelength of 540 nm. Fruit Ca was determined by an induction couplet plasma spectrometer. All nutrient concentrations were expressed as mg kg$^{-1}$ fresh weight (FW).

### *Weather conditions*

Summer 2002 was warmer with less precipitation than the average of many years in Estonia. The mean

Table I. Nutrient content of the soil in apple orchard in different experimental variants during 2002–2003. The asterisks (*) indicate nutrient content compared to the sufficient nutrient levels recommended for apples in Estonia: * low, ** average, *** high, **** very high.

| Experimental variant | P mg kg$^{-1}$ | K mg kg$^{-1}$ | Ca mg kg$^{-1}$ | Mg mg kg$^{-1}$ | $pH_{KCl}$ |
|---|---|---|---|---|---|
| Control | 176**** | 225*** | 1067** | 70.5** | 5.66 |
| Calcium treated | 129*** | 206*** | 1061** | 67.5** | 5.64 |

air temperature was especially higher than the average for July and August (Table II). At the same time precipitation was extremely low in May, when only 15.4 mm of rain fell, compared to an average of 55 mm for this time period. Precipitation was also very low in July, August and September.

In summer 2003 air temperature was approximately the same as the averages for May, August and September; June was warmer and July cooler than the average. At the same time summer was very wet; an especially large amount of precipitation was recorded in May, July and August. However, before harvest in September, the weather was relatively dry.

*Statistical methods*

To study the influence of calcium treatment on apple mineral composition, spoilage and physiological disorders, analysis of variance was used. The effect of calcium treatment is presented in the figures and the mean values to be compared are followed by the same letter if they are not significantly different at $p \leq 0.05$. To study correlation between fruit mineral nutrient content, fruit spoilage and disorders, correlation analysis was used. Linear correlation coefficients between variables were calculated, the significance of coefficients being $p \leq 0.001$***, $p \leq 0.01$**, $p \leq 0.05$*, ns = non-significant.

## Results

*Loss of marketable yield and incidence of physiological disorders*

In the first storage season the effect of calcium treatment on the percentage of spoiled apples was not significant in either cultivar (data not shown). In the second storage season first loss of marketable yield was noticed in 'Krameri Tuviõun' after two months of storage. Calcium treatment significantly reduced spoilage. The effect remained significant until the end of storage, reducing the number of spoiled apples of 'Krameri Tuviõun' by 33% (Figure 1). After four months of storage, fruits of

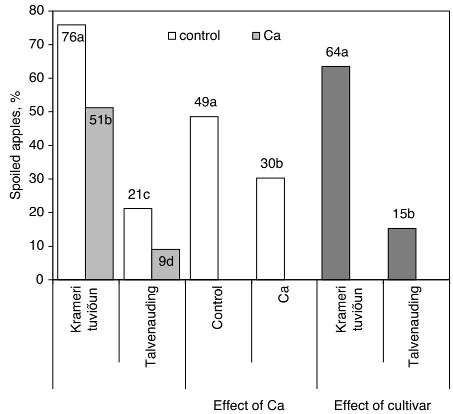


Figure 1. The percentage of spoiled apples after four months of storage as affected by pre-harvest calcium treatment in second experimental year.

'Talvenauding' started to suffer from superficial scald. The effect of calcium treatment was significant, reducing the number of spoiled apples by 57%. At the end of the storage period the effect of calcium treatment on spoilage of 'Talvenauding' fruits was no longer significant.

Bitter pit incidence was not affected by calcium treatment in either of the experimental years. Physiological spot had a different response: in the first year calcium treatment significantly increased and in the second year significantly reduced the number of fruit with physiological spot.

In the first year superficial scald incidence was not significantly influenced by calcium treatment, but in second year treatment significantly reduced the number of fruit affected by this disorder.

*Content of fruit nutrients and their balance after harvest*

In the first experimental year fruit Ca content ranged from 35.4 to 46.5 mg kg$^{-1}$ FW (Figure 2). The average Ca content in 'Talvenauding' fruits was 24% higher than in 'Krameri Tuviõun' fruits. Calcium

Table II. Weather conditions in summer 2002 and 2003 in south Estonia: monthly mean air temperature, °C and mean monthly precipitation, mm compared to the same figures for 1966–1998 in Estonia.

| | 2002 | | 2003 | | 1966–1998 | |
|---|---|---|---|---|---|---|
| | Temp. | Precip. | Temp. | Precip. | Temp. | Precip. |
| May | 13.9 | 15.4 | 11.7 | 142.8 | 11.0 | 55 |
| June | 16.5 | 50.1 | 12.9 | 71.2 | 15.1 | 66 |
| July | 20.1 | 44.7 | 19.4 | 103.6 | 16.7 | 72 |
| August | 19.2 | 22.2 | 15.2 | 132.8 | 15.6 | 79 |
| September | 12.1 | 20.0 | 11.4 | 24.6 | 10.4 | 66 |

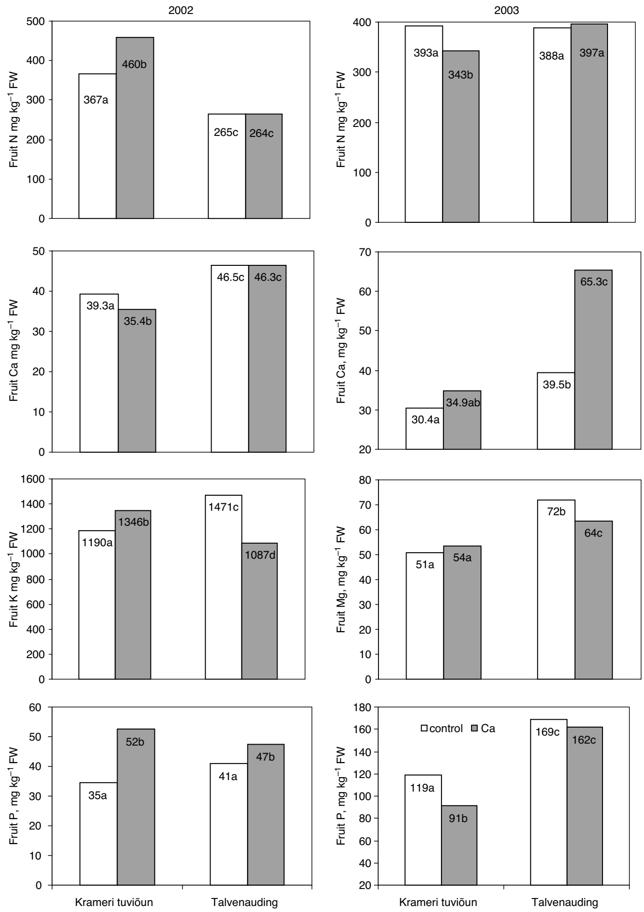


Figure 2. Content of fruit N and Ca, mg kg$^{-1}$ FW after harvest in 2002 and 2003; fruit K after harvest in 2002 and fruit Mg after harvest in 2003 as affected by pre-harvest calcium treatment of apples in Estonia.



treatment reduced Ca content in 'Krameri Tuviõun' and had no influence on 'Talvenauding'. In the second experimental year fruit Ca content ranged from 30.4 to 65.3 mg kg$^{-1}$ FW (Figure 2). Ca content of treated 'Talvenauding' increased significantly, being 60% higher on average compared to 'Krameri Tuviõun'.

In the first experimental year fruit N content ranged from 264 to 460 mg kg$^{-1}$ FW. The content of N in 'Krameri Tuviõun' was higher than in 'Talvenauding'. Calcium treatment significantly increased N content in 'Krameri Tuviõun', but did not influence N content in 'Talvenauding' fruits. In the second season fruit N content ranged from 343 to 397 mg kg$^{-1}$ FW. Calcium treatment significantly reduced N content in 'Krameri Tuviõun' apples.

In the first experimental year fruit K content ranged from 1087 to 1471 mg kg$^{-1}$ FW (Figure 2). Calcium treatment influenced both cultivars, but differently: K content in 'Krameri Tuviõun' fruits increased and in 'Talvenauding' fruits decreased. In the second experimental year fruit K content ranged from 1115 to 1748 mg kg$^{-1}$ FW. The effect of calcium treatment was not significant in any of the variants (data not shown).

In the first experimental year fruit Mg content ranged from 9.9 to 12.5 mg kg$^{-1}$ FW. Mg content in apples was not influenced either by calcium treatment or cultivar (data not included). In the second experimental year fruit Mg content ranged from 50.9 to 71.9 mg $kg^{-1}$ FW (Figure 2). Calcium treatment significantly increased Mg content in 'Talvenauding' fruits, but did not have an effect on 'Krameri Tuviõun'.

Fruit P content ranged from 35 to 53 mg $kg^{-1}$ FW in 2002 (Figure 2). Calcium treatment increased P content in fruits of both cultivars. In 2003 fruit P content ranged from 91 to 169 mg $kg^{-1}$ FW (Figure 3). Calcium treatment reduced significantly P content in 'Krameri Tuviõun'. Cultivar differences were also significant, and P content in 'Talvenauding' fruits was higher.

The N/Ca ratio in fruits was similar in both years, remaining between 6 and 13 (Figure 3). In 2002 calcium treatment increased N/Ca ratio in 'Krameri Tuviõun'; N/Ca ratio in apples was almost double compared to 'Talvenauding'. In 2003 calcium treatment affected both cultivars, reducing the N/Ca ratio in apples by an average of 31%. As with the previous year, the N/Ca ratio in 'Talvenauding' was lower.

The average K/Ca ratio in apples was 30.9 and 34.5 in 2002 and 2003, respectively (Figure 3). In both years calcium treatment significantly reduced the K/Ca ratio in 'Talvenauding'. The K/Ca ratio in'Krameri Tuviõun' was significantly increased in 2002 and was not influenced in 2003.

The Mg/Ca ratio in apples differed annually, ranging from 0.20 to 0.35 in 2002 and from 1.0 to 1.8 in 2003 (Figure 3). The Mg/Ca ratio of 'Krameri Tuviõun' was not affected by calcium treatment in either year, while the Mg/Ca ratio in 'Talvenauding' in 2002 was increased and in 2003 decreased by calcium treatment.

### *Correlations between fruit nutrients, loss of marketable yield and physiological disorders*

In both experimental years the percentage of spoiled fruits after four months of storage correlated negatively with fruit Ca content and positively with N/Ca balance in the fruits (Table III). Other correlations differed annually. In the first year fruit spoilage after

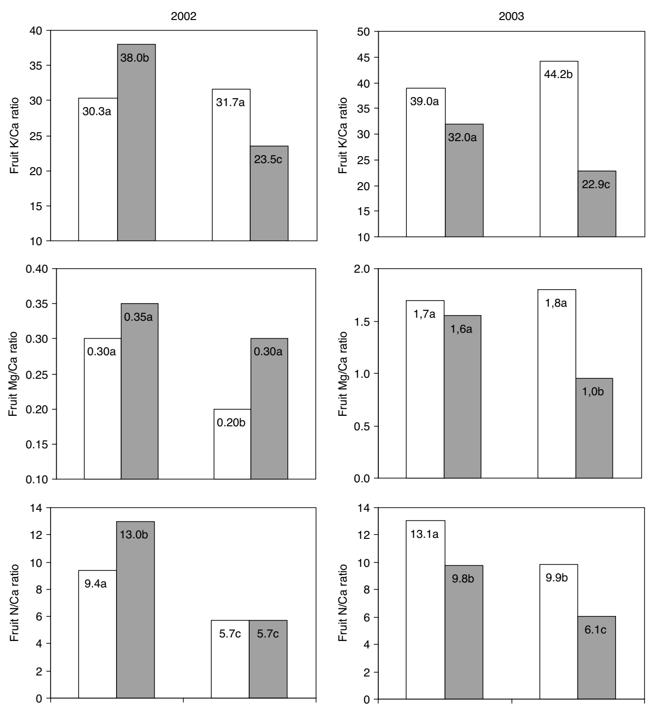


Figure 3. Content of fruit P, mg $kg^{-1}$ FW and K/Ca and Mg/Ca ratio after harvest in 2002 and 2003 as affected by pre-harvest calcium treatment of apples in Estonia.

Table III. Correlation coefficients (*r*) between apple fruit mineral content after harvest, percentage of spoiled fruits four months after storage in different years and incidence of physiological disorders (percentage of affected fruits) as an average of two experimental years after four months of storage in Estonia. The asterisks (*) indicate significance levels: $*p \leq 0.05$, $**p \leq 0.01$, ns = not significant.

| Fruit mineral content, mg kg$^{-1}$ FW | Spoiled fruits in first year | Spoiled fruits in second year | Bitter pit | Physiological spot | Superficial scald |
|---|---|---|---|---|---|
| Ca | −0.957** | −0.797* | 0.761** | 0.257 ns | −0.589* |
| K | 0.029 ns | −0.752* | 0.476 ns | 0.861** | 0.193 ns |
| Mg | 0.402 ns | −0.824** | −0.893** | −0.771** | 0.293 ns |
| P | 0.015 ns | −0.733* | −0.883** | −0.520* | 0.249 ns |
| N | 0.926** | −0.215 ns | 0.253 ns | 0.769** | 0.172 ns |
| K/Ca | 0.697 ns | 0.321 ns | −0.371 ns | 0.280 ns | 0.551* |
| N/Ca | 0.922** | 0.839** | −0.310 ns | 0.373 ns | 0.652** |
| Mg/Ca | 0.811* | 0.444 ns | −0.897** | −0.719** | 0.511* |

four storage months showed a significant positive correlation with N content and Mg/Ca ratio in fruits. By the end of the six months' storage period, no correlations were found between spoilage and fruit mineral composition. In the second year fruit spoilage after four months of storage had a significant negative correlation with K, Mg and P content.

Regarding the different physiological disorders, correlations are different from general correlations with loss of marketable yield. As an average of two years observations, superficial scald of 'Talvenauding' correlated negatively with Ca content and positively with K/Ca ratio, N/Ca ratio and Mg/Ca ratio in fruits (Table III). Bitter pit in 'Krameri Tuviõun' correlated positively with Ca content and negatively with Mg and P content and Mg/Ca ratio in apples. Physiological spot on the same apple had a significant positive correlation with apple K and N content and a negative correlation with Mg and P content and Mg/Ca ratio in apples.

## Discussion

In the first experimental year calcium treatment did not significantly reduce any physiological disorders or loss of marketable yield of apples. During this year incidence of physiological disorders was generally high. One reason could be drought and high air temperatures during the two months prior to harvesting (Table II). Earlier studies in Estonia (Lamp, 1981), in Finland (Dris et al., 1998) and in Chile (Yuri, 1995) have shown that warm vegetation periods and drier seasons favour the development of physiological disorders in apples.

Generally, fruit Ca content in our study was lower than the recommended amount of 50 mg kg$^{-1}$ FW (Sharples, 1980). In the second experimental year only fruits from calcium-treated 'Talvenauding' variant reached the value of 65 mg kg$^{-1}$ FW, which is as high as recommended for potential good keeping quality. The response was seen on apple post-harvest quality, since spoilage of 'Talvenauding' dropped significantly after four months of storage. Based on these results, we may say that superficial scald disorder in 'Talvenauding' is related to mineral nutrition, which has not been stated before. Correlations between superficial scald and fruit mineral composition indicate that content of Ca and its ratios with K, N and Mg play an important role in severity of scald, and that increasing fruit Ca by preharvest calcium sprays can be recommended for improving post-harvest quality of 'Talvenauding'.

Fruit N content was also low in both years when compared to the recommended level of 500–700 mg kg$^{-1}$ FW (Sharples, 1980). Since fruit Ca content did not increase significantly during the first experimental year, but fruit N content increased, it can be suggested that despite the relatively low N level, the increase in N content still had a harmful impact on apple post-harvest quality, probably by increasing respiration rate and ethylene production.

Fruit N/Ca level was influenced mostly by cultivar: average N/Ca ratio in 'Krameri Tuviõun' fruits was higher (11.4 in both years) than in 'Talvenauding' fruits (5.7 in 2002 and 8.0 in 2003). Based on that ratio, 'Krameri Tuviõun' could have better storage potential, because for good quality apples, the N/Ca ratio should be either about 10 (Shear, 1974), or ranging from 10 to 14 (Sharples, 1980; Dris, 1998). In our study neither bitter pit nor physiological spot in 'Krameri Tuviõun' showed a correlation with the N/Ca ratio, which seems to confirm our previous statements, and indicates that these particular disorders are affected by other nutrients than Ca and N. Superficial scald in 'Talvenauding' correlated positively with N/Ca ratio, indicating that the recommended N/Ca ratio is too high for 'Talvenauding'.

The adequate range for fruit potassium is said to be between 1300 and 1600 mg kg$^{-1}$ FW, fruit P above 110 mg kg$^{-1}$ FW, and fruit Mg about 50 mg kg$^{-1}$ FW (Sharples, 1980). Thus in the first experimental year fruit K, P and Mg content in apples was too low and in the second year it was sufficient.

Despite this, in the second experimental year general fruit spoilage had a negative correlation with all of these nutrients, indicating that for good keeping quality, content of these nutrients should be higher in these cultivars.

Both physiological disorders of 'Krameri Tuviõun' correlated negatively with Mg and P content and Mg/Ca ratio in apples (Table III). Since bitter pit in 'Krameri Tuviõun' correlated positively with Ca content, the benefit of calcium treatment in this cultivar is doubtful, as Ca and Mg are known to be antagonistic in fruit nutrition. Yuri et al. (2002) also found that subepidermal Ca levels at harvest explained only 53.3% of the variability in external bitter pit incidence after 120 days of storage. Retamales and Valdes (2001) stated that infiltration of Mg into fruit would provide a more reliable forecast. Even though numerous studies have confirmed that calcium treatment reduces bitter pit (Reid & Padfield, 1975; Neilsen & Neilsen, 2002), our results did not support these findings. Although in the second year the number of spoiled fruits of 'Krameri Tuviõun' decreased (Figure 1), it was as a result of the reduction of physiological spot, not bitter pit. Recently, it has been proposed that bitter pit is essentially the result of a gibberellin-induced increased susceptibility of the cell membranes to stress, and calcium only reduces the effect of gibberellins (Saure, 2002). Therefore it would be worth trying other measures for this cultivar, which would either interfere with gibberellin biosynthesis, or promote production of endogenous antagonists of gibberellins.

Both of our hypotheses were verified. In southern regions the main benefit of calcium treatment is decreasing bitter bit incidence; but in our experiment bitter pit in 'Krameri Tuviõun' was not reduced by calcium treatment; instead it correlated negatively with Mg and P content and Mg/Ca ratio in apples. According to the second hypothesis, calcium treatment affects other apple physiological disorders, such as reducing superficial scald and physiological spot. A conclusion can be made that pre-harvest calcium treatment has an effect on mineral composition of apples in the Nordic region. Content of Ca and its ratios with other nutrients plays an important role in the development of superficial scald on 'Talvenauding' fruits, and pre-harvest calcium sprays can be recommended for improving post-harvest quality of this cultivar.

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# II

**Mainla, L.**, Noormets, M., Moor, U., Karp, K., Jalakas, M. 2011.
EFFECT OF ROOTSTOCK ON TASTE-RELATED PROPERTIES
OF NORDIC APPLE CULTIVARS.
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# Effect of Rootstock on Taste-Related Properties of Nordic Apple Cultivars

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**Keywords:** *Malus* × *domestica*, M.26, M.9, B.396, ascorbic acid, titratable acidity, soluble solids

**Abstract**

**The effect of rootstock on taste-related properties of four Nordic apple (*Malus* × *domestica* Borkh.) summer cultivars ('Valge Klaarõun', 'Maikki', 'Pirja', 'Krasnoje Rannjaja') grafted on three different rootstocks (M.26, M.9, B.396) was studied. The experiment was carried out in South Estonia in 2003 and 2004. Rootstock had a significant effect on apple titratable acidity (TA) in both years. In 2003, B.396 decreased TA in 'Pirja', ′Krasnoje Rannjaja' and 'Maikki' apples. In both years rootstock had the same effect only on 'Valge Klaarõun' where B.396 increased TA content in apples. In 2003 rootstock had a significant effect on soluble solids content (SSC) only on 'Valge Klaarõun' where M.26 increased significantly fruit SSC. In the second year fruits of 'Maikki' on M.9 had significantly lower SSC. SSC and TA ratio ranged from 5 to 30. In 2003 rootstock had a significant effect on SSC/TA in fruits of all cultivars. In 2004 no significant effect was observed except on 'Maikki'. In 'Valge Klaarõun' fruits the SSC/TA was significantly higher with M.26. The mean ascorbic acid content (AAC) in apples was 19.3 mg 100 g$^{-1}$ FW in 2003 and 11.6 mg 100 g$^{-1}$ FW in 2004. In both years rootstock had no significant effect on fruit AAC of 'Maikki'. 'Krasnoje Rannjaja' grafted on M.9 had significantly higher fruit AAC in 2003. 'Valge Klaarõun' apples grafted on M.26 had increased AAC.**

## INTRODUCTION

Low temperatures in winter, large temperature fluctuations in early spring and a short vegetative period are the main problems of apple growers in Nordic Countries. In addition to winter hardiness the influence of rootstock on apple quality is also important. In grafted fruit trees, the tree growth and yield and fruit quality is influenced by both the rootstock and the scion cultivar (Skrzynski, 2007; Rubauskis and Skrivele, 2007). Storage experiments have shown that the type of rootstock can influence the quality of apple after storage (Skrzynski, 2007).

Studies of fruit quality have found relationships between °Brix levels, titratable acidity, °Brix/titratable acidity ratio and consumer acceptability (Vangdal, 1985; Harker et al., 2001). Consumers are also interested in health benefits of apples. Vitamin C (ascorbic acid) is an important antioxidant (Padayatty et al., 2003). Therefore it is important to study, in addition to the influence of rootstocks on winter hardiness and yield, the effect on the biochemical quality parameters of apples. The goal of this experiment was to study the influence of rootstocks B.396, M.26 and M.9 on fruit quality of four Nordic apple cultivars 'Valge Klaarõun', 'Maikki', 'Pirja' and 'Krasnoje Rannjaja'.

## MATERIALS AND METHODS

The experiment was carried out in South Estonia (58°21' N, 26°31' E) in 2003 and 2004. Four Nordic summer cultivars 'Valge Klaarõun' (Baltic), 'Maikki' (Finland), 'Pirja' (Finland) and 'Krasnoje Rannjaja' (Russia) on rootstocks M.26, M.9 and B.396 were planted in spring 2001 with distance 2 m between the trees and 4 m between the rows. Trees were planted in a randomized complete block design with four replicates and four trees per plot. The trees were trained as a spindle and were not irrigated. The ground between the rows was grassed and along the rows treated with herbicides. Plants were only fertilized during planting with (kg $ha^{-1}$): N 50, P 20 and K 40. 'Valge Klaarõun' on M.9 had no apples in 2003 therefore it was left out in the first year.

In 2003 the air temperature in July was approximately the same as the average of many years (18°C). June (13°C) and August (15°C) were cooler than the average (15°C and 17°C respectively). The summer was very rainy; especially in July (104 mm) and August (133 mm). In 2004 the air temperature in August was approximately the same as the average of many years. Both June and July were cooler than the average. July (76 mm) was dryer than the average (80 mm), but June (184 mm) and August (105 mm) were very rainy. Especially in June, when it rained 2.5 times more than normal.

Ten fruits from each cultivar from each treatment and replicate were taken for the fruit quality analyses. Apples were sliced, core removed and both skin and flesh tissue were used for all analyses.

Titratable acidity (TA) was determined by taking 5 g from ground samples, put into 100 ml tubes, which were filled with 80°C distilled water. The dilutions were heated for two hours in 80°C, then cooled down to the room temperature, filtered and titrated with 0.1 N NaOH until pH 8.2. TA was expressed as g malic acid·100 $g^{-1}$ FW.

For determination of ascorbic acid content (AAC) a sample of 10 g was crushed quickly for each analysis. Iodometric determination method M167 (www.mt.com) was used with modification: instead of using sulphuric acid, 60 ml of a mixture of metaphosphoric and acetic acid (3% $HPO_3$ + 8% $CH_3COOH$) was added instantly to avoid vitamin C breakdown in air (Paim and Reis, 2000). Titrator Mettler Toledo DL50 with autosampler Rondolino was used for titration of AAC and TA.

Soluble solids content (SSC) was measured using digital refractometer ATAGO CO., Ltd., Japan. SSC was expressed as °Brix.

Data were analyzed by one way analysis of variance. Effect of rootstock was evaluated separately for each cultivar. The mean comparisons were evaluated at $P \leq 0.05$.

**RESULTS**

The AAC varied among the years. In 2003 fruits had higher content (from 14 to 24 mg·100 $g^{-1}$ FW) and lower (from 8 to 15 mg 100 $g^{-1}$ FW) in 2004 (Fig. 1). In both years rootstock had no significant effect on fruit AAC of 'Maikki'. However, 'Krasnoje Rannjaja' grafted on M.9 had significantly higher fruit AAC in 2003 than with other rootstocks. With 'Valge Klaarõun', M.26 had increased AAC compared to the other stocks. The effect was significant only in the first year, but in 2004 the same tendency could be seen. With 'Pirja', trees on M.26 had reduced AAC in fruits in 2003, but in 2004 rootstocks had no significant influence.

TA content was very constant. In 2003 TA content ranged from 0.5 to 1.0 g·100 $g^{-1}$ FW, being higher than in 2004, when it ranged from 0.4 to 0.6 g·100 $g^{-1}$ FW (Fig. 2). Rootstock had a significant effect on all cultivars in both years. In 2003 B.396 had reduced TA in 'Pirja', 'Krasnoje Rannjaja' and 'Maikki' apples. In both years rootstock had the same effect only on 'Valge Klaarõun' where B.396 increased TA content. In 2003 trees on M.9 had increased TA content in apples of 'Maikki', but in 2004 it had reduced TA. The same effect was seen with M.26 with 'Pirja' fruits. In our 2-year experiment TA content in 'Pirja' fruits was similar in both years.

In 2003 SSC in apples ranged from 6 to 9°Brix and in 2004 from 9 to 11°Brix (Fig. 3). In 2003 rootstock had a significant effect only on 'Valge Klaarõun' where M.26 significantly increased fruit SSC. In 2004 the same tendency could be seen, but the trend was not significant. In the second year rootstock had a significant influence on SSC of 'Maikki' where fruits from trees on M.9 had significantly lower SSC. Rootstocks had no significant effect on apples SSC in 'Krasnoje Rannjaja' and 'Pirja' in either year.

SSC and TA ratio ranged from 5 to 30 (Fig. 4). The ratio was higher in the second experimental year because of higher SSC and lower TA content in apples. In 2003 rootstocks had a significant effect on SSC/TA in fruits of all cultivars. In 2004 there was a significant effect only with 'Maikki'. With 'Valge Klaarõun' fruits from trees on M.26

had significantly higher SSC/TA with M.26.

## DISCUSSION

Rootstocks had significant effect on AAC in ‘Krasnoje Rannjaja’ fruits in both years, but the influence was different in different years. Thus, we can not recommend these rootstocks for increasing fruit AAC. The effect of rootstocks was dependent on the weather conditions in different years. The AAC in fruits is influenced by the temperature and light (Lee and Kader, 2000). In 2003 the AAC in apples was higher than in 2004. The main factor for that might explain this difference is the warmer and wetter July than in 2004. AAC in widely known to be variable in commercial apple cultivars: 12.8 mg·100 $g^{-1}$ FW was recorded in a study of six apple cultivars by Lee et al. (2003) and 5.7 mg·100 $g^{-1}$ FW by Eberhardt et al. (2003). In our study the AAC in apples was higher in 2003, mean AAC in fruits was 19.3 mg·100 $g^{-1}$ FW.

SSC and TA are the most common parameters measured in fruit quality analyses, and a change in the ratio between these parameters can have an impact on the taste of the apple. Earlier studies have shown that the best objective predictor of sweetness is SSC, which could predict a difference in taste when apple SSC differed by more than 1% (Harker et al., 2001). In both experimental years rootstocks had a significant effect on SSC in ‘Valge Klaarõun’ apples with sweeter apples from trees on M.26. In other studies fruits from different cultivars grown on M.9 had higher SSC (Kviklys and Kvikliene, 2002; Skrzynski and Gastol, 2007). In our experiment M.9 caused significantly lower SSC in fruits of ‘Maikki’ and ‘Valge Klaarõun’ in 2004. Thus, the effect of rootstock is dependent also on the cultivar and growing site as concluded by Autio et al. (2001). SSC varied from year to year in ‘Gala’ apple fruits (Rutkowski et al., 2005). SSC in apples also varied among the years in our experiment and was higher in 2004.

Titratable acidity has proven to be the best predictor of acid taste and differences of 0.08% titratable acidity between apples could evoke a response in perceived acid taste (Harker et al., 2001). Therefore rootstocks had a significant effect on the acid taste. The influence of rootstock on apples acid taste was dependent on cultivar. Only with ‘Valge Klaarõun’ was the effect in both years was the same where fruit from trees on B.396 apples had higher TA than with M.9 or M.26. Daugaard and Callesen (2002) found that the highest TA in ‘Mutsu’ fruits was on M.26, however considerable variation among years was also recorded. Skrzynski and Gastol (2006) also found that TA was influenced by the rootstock type and the year of study.

## CONCLUSIONS

The influence of dwarf rootstocks on apple quality was significant, but the effect of rootstock was different in different years and was also influenced by cultivar and different weather conditions. Therefore, based on our results, none of the three rootstocks we tested can be preferred in order to alter the taste or AAC of apples. Only in with the cultivar ‘Valge Klaarõun’ were there consistently sweeter apples when grown on M.26.

## ACKNOWLEDGEMENTS

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**Figures**

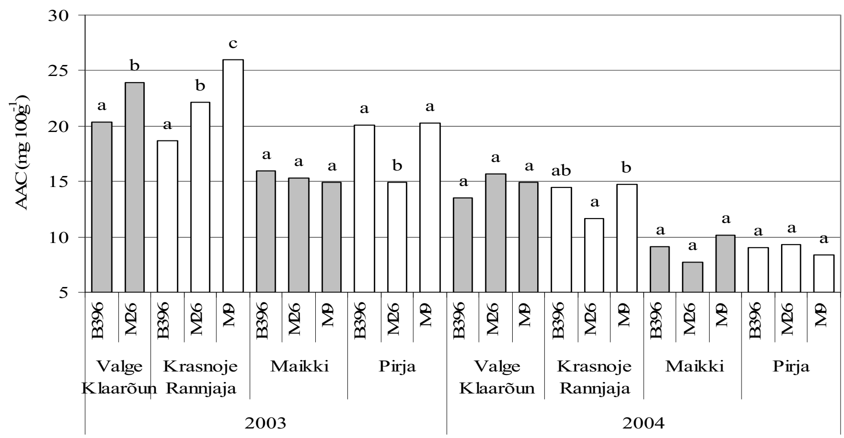


Fig. 1. Influence of rootstock on apple ascorbic acid content. Means followed by different letters are significantly different (p=0.05).

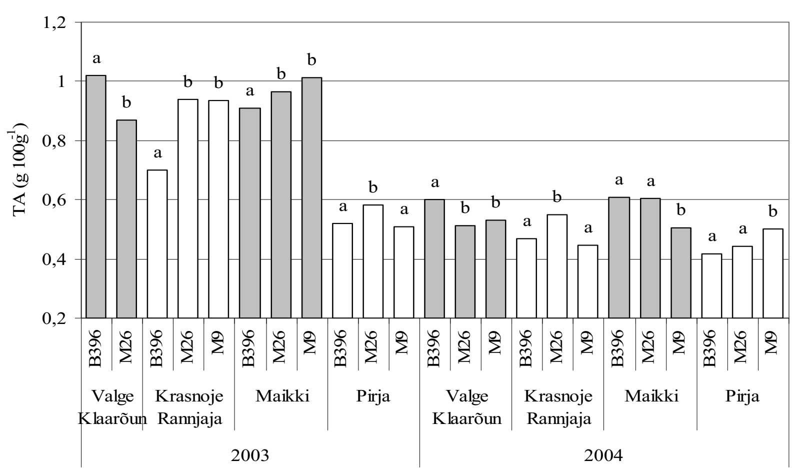


Fig. 2. Influence of rootstock on apple titratable acidity. Means followed by different letters are significantly different (p=0.05).

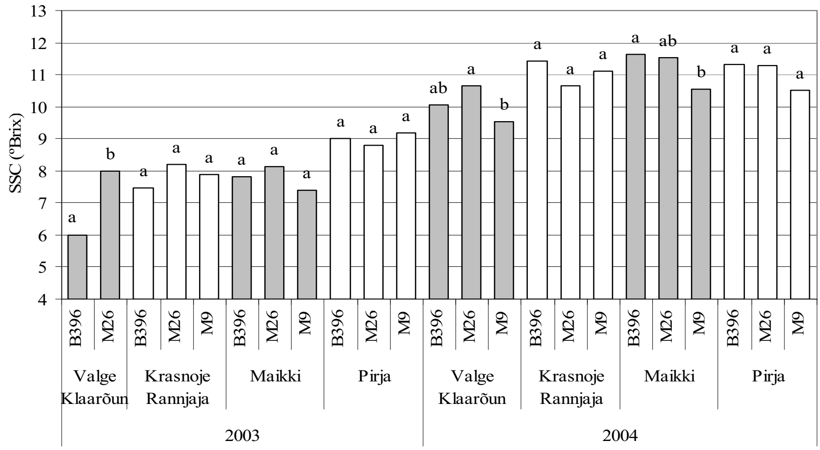


Fig. 3. Influence of rootstock on apple soluble solids content. Means followed by different letters are significantly different (p=0.05).

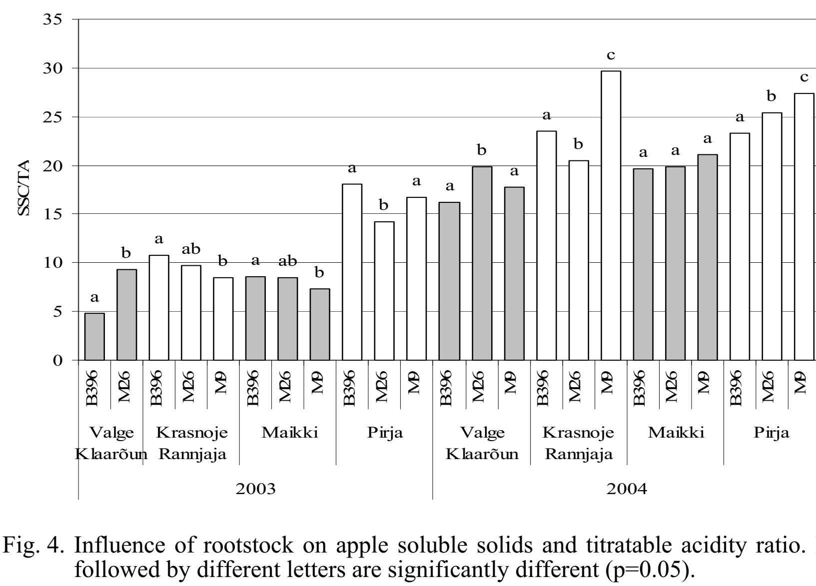


Fig. 4. Influence of rootstock on apple soluble solids and titratable acidity ratio. Means followed by different letters are significantly different (p=0.05).

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# III

**Mainla, L.**, Moor, U., Karp, K., Püssa, T. 2011.
THE EFFECT OF GENOTYPE AND ROOTSTOCK ON POLYPHENOL COMPOSITION OF SELECTED APPLE CULTIVARS IN ESTONIA.
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# The effect of genotype and rootstock on polyphenol composition of selected apple cultivars in Estonia

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## Abstract

The aim of the two year study, 2006 and 2007, was to find out the concentration of the main polyphenols and the estimated content of total polyphenols in the peel of six apple (*Malus* x *domestica* Borkh.) cultivars: 'Talvenauding', 'Krista', 'Liivi Kuldrenett', 'Lobo', 'Cortland' and 'Antei', grown in Estonia. Additionally, the effect of rootstocks B.396, M.26 and 'Antonovka' seedling on the content of polyphenols of 'Talvenauding' apples was studied. High-performance liquid chromatography (HPLC) was used to quantify the polyphenols: catechin, chlorogenic acid, phloridzin, quercetin, quercetin galactoside and quercitrin, all identified by tandem mass spectrometry (MS/MS).

The content of polyphenols was affected by the genotype, rootstock and also by the weather conditions of the particular year. The largest variation of concentrations between the cultivars occurred in quercetin and quercetin galactoside. 'Krista' and 'Lobo' had a 23-fold difference in quercetin concentration being 117.6 mg 100 $g^{-1}$ FW and 5.8 mg 100 $g^{-1}$ FW, respectively. Polyphenols in the apple peel occurred in the following decreasing order: phloridzin > quercitrin > catechin > chlorogenic acid > quercetin galactoside > quercetin and this order was not affected by the genotype. The cultivars with higher concentration of catechin and flavonols had also higher estimated content of total polyphenols. Among the studied rootstocks, the concentration of polyphenols in 'Talvenauding' apples was higher when vegetative rootstocks were used compared to the seedling rootstock.

Keywords: *Malus* x *domestica*, catechin, chlorogenic acid, phloridzin, flavonols, HPLC.

## Introduction

Over the past two decades studies *in vitro* and *in vivo* have shown the beneficial effect of fruits and vegetables on human health (Wang et al., 1997; Joshipura et al., 1999). Much of the protective effect has been attributed to phytochemicals, which are non-nutrient plant compounds such as carotenoids, flavonoids, isoflavonoids and phenolic acids (Kampa et al., 2004). Apples are a rich source of phytochemicals, and epidemiological studies have linked the consumption of apples with reduced risk of some cancers, cardiovascular disease, asthma and type II diabetes (Knekt et al., 2002).

Hydroxycinnamic acid derivatives (chlorogenic acid), flavonols (quercetin glycosides), flavanols (catechin, epicatechin, procyanidins) and dihydrochalcones (phloridizin) are the major phenolics in apples (Lee et al., 2003). The distribution of phenolic compounds varies considerably among different cultivars and also within different tissues (Khanizadeh et al., 2008). Anthocyanins and quercetin glycosides exist exclusively in the apple peel whereas epicatechin, procyanidins, phloridzin and chlorogenic acid are found in both peel and flesh of apples (Hagen et al., 2007). Since the apple peel contains more antioxidant compounds, especially quercetin, the peel may have higher antioxidant activity and higher bioactivity than the flesh (Eberhardt et al., 2000).

According to the reported data the important factors influencing the content of polyphenols in the apple are: cultivar properties, fruit maturity, weather conditions of the harvesting season, processing, agricultural conditions, crop load, development of infection, fruit position within the canopy and geographic location (Awad et al., 2000; Van der Sluis et al., 2001). The effect of rootstock is less discussed. Scalzo et al. (2005) have found that the rootstocks in apricots and peaches play an important role in determining the total amount of phenolic compounds. On the other hand, considering factors influencing apple quality, the list also includes rootstocks. From the health point of view the concentration of polyphenols is considered as an important constituent of apple quality. Hence the influence of rootstock should also be taken into account as an important factor influencing the concentration of polyphenols.

Phenolic compounds are induced in plants by various biotic and abiotic stresses (Dixon, Paiva, 1995). Cold treatments and drought stress cause increases in levels of (-)-epicatechin and quercetin-3-galactoside in *Crataegus laevigata* and *C. monogyna*. These types of treatments also enhance the antioxidant capacity of the shoot extracts, and may be the primary function of these cold-inducible flavonoids (Kirakosyan et al., 2003). Marais et al. (2001) have found that a fluctuating temperature resulted in better colour and higher anthocyanin concentrations for apple fruits harvested from different areas.

For many years, the important goals in apple research in Nordic climate have been finding out cultivars and rootstocks with good winter hardiness and appealing appearance. However, yield quality in respect to the health-beneficial compounds is becoming increasingly important every year. Data about phenolic content in Nordic apple cultivars is almost non-existent and information about the effect of rootstock on polyphenol content is also absent. It could be assumed that the content of polyphenols could be increased by choosing the suitable rootstock and cultivar combination, but there is no data available weather the influence is consistent in different years.

The aim of the two year study, 2006 and 2007, was to find out the concentration of the main polyphenols and the estimated content of total polyphenols in the peel of six apple (*Malus* x *domestica* Borkh.) cultivars: ‘Talvenauding’, ‘Krista’, ‘Liivi Kuldrenett’, ‘Lobo’, ‘Cortland’ and ‘Antei’, grown in Estonia. Additionally, the effect of rootstocks B.396, M.26 and ‘Antonovka’ seedling on the content of polyphenols in the peel of ‘Talvenauding’ apples was studied.

## Materials and methods

*Plant material and agricultural practices.* The experiment was carried out in 2006 and 2007 at the Estonian University of Life Sciences’ Rõhu Research Center (58°21′ N, 26°31′ E) located in South Estonia. The apple trees for cultivar testing were grafted onto ‘Antonovka’ seedlings and planted in 1986 with a distance of 4 m between the trees and 8 m between the rows.

The experiment’s cultivars (and country of origin) were: ‘Talvenauding’ (Estonia), ‘Krista’ (Estonia), ‘Liivi Kuldrenett’ (Baltic), ‘Lobo’ (Canada), ‘Cortland’ (USA) and ‘Antei’ (Belarus).

The trees for rootstock testing were planted in 2001 with 2 m between the trees and 4 m between the rows. ‘Talvenauding’ was grafted onto semi-dwarfing rootstock M.26 (England), dwarfing rootstock B.396 (Russia) and vigorous rootstock ‘Antonovka’ (Russia) seedling.

The ground between the rows was grassed and the rows were treated with herbicides. Soil analysis of the vegetative rootstock experiment orchard showed: $pH_{KCl}$ 7.0, P – 175 mg $kg^{-1}$, K – 197 mg $kg^{-1}$, Ca – 2980 mg $kg^{-1}$ and humus – 4.7%. Soil analyses for the cultivar testing orchard showed: $pH_{KCl}$ 6.3, P – 216 mg $kg^{-1}$, K – 236 mg $kg^{-1}$, Ca – 2830 mg $kg^{-1}$ and humus 5.9%. Since the soil analyses showed no nutrient deficiencies and the trees were growing well, no mineral fertilizers were used at the experimental plantation in either of the experimental years. For plant protection, the apple trees were sprayed four times during the vegetation period to prevent apple scab using copper oxide chloride at the end of April, cypprodinil in the middle of May, difenoconazole at the end of June and ditianon in the middle of July.

*Weather conditions.* According to agro climatic regions of Estonia, and also taking into account several studies of microclimate, Kask (2000) has divided Estonia into different regions based on their suitability for fruit production. Rõhu is situated in the Tartu region, where cold winters with very low temperatures (sometimes below −37ºC, in certain areas −39ºC) are typical. A vegetation period free of night frosts is short (130 days) and night frosts may be severe. Snow falls quite late and severe frosts in November without snow cover (below −20ºC or even more on the ground) may damage fruit crops.

In the current experiment, the weather during May, June and July in 2006 was fairly dry as only 49% of the average precipitation for these three months fell (Fig. 1). Only in August 2006, the precipitation was more than the average for this period. Conversely in 2007, the precipitation for May was double the average and the driest month was August. In 2006, the mean air temperature was above the average, being especially high in July: 18.5ºC, (average 16.7ºC) and September 13.1ºC (average 10.4ºC). The mean air temperature in 2007 was close to the average except for May 13.6ºC (average 11.0ºC) and August 17.7ºC (average 15.6ºC).

The duration of sunlight hours in 2006 was longer in July and September and shorter in August than the average, but in 2007 was longer in June and August and shorter in July and September compared to the average.

*Polyphenol analysis.* In both years 'Krista' and 'Liivi Kuldrenett' apples were harvested in mid-September, 'Antei', 'Talvenauding', 'Cortland' and 'Lobo' in the third ten-day period of September. Samples of 50 first quality fruits per cultivar were picked from the outer periphery of the canopy. Fruits were stored at between 2 and 5ºC and 90… 95% relative humidity in a normal atmosphere storehouse for three months. The analyses of polyphenols were performed on the ripe apples at the end of November.

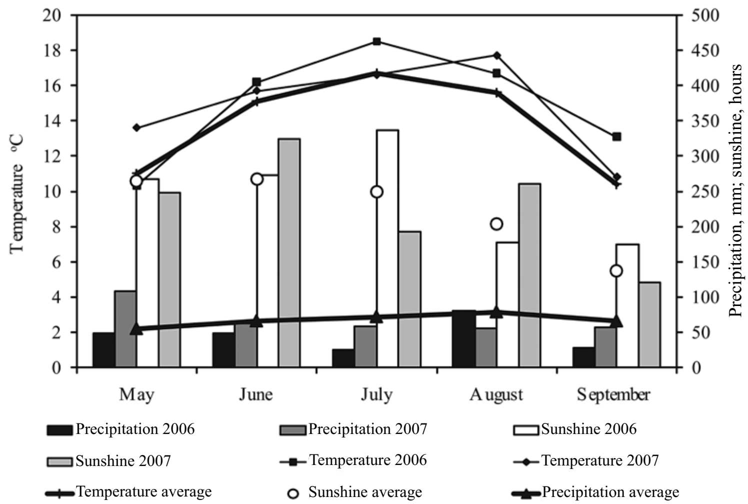
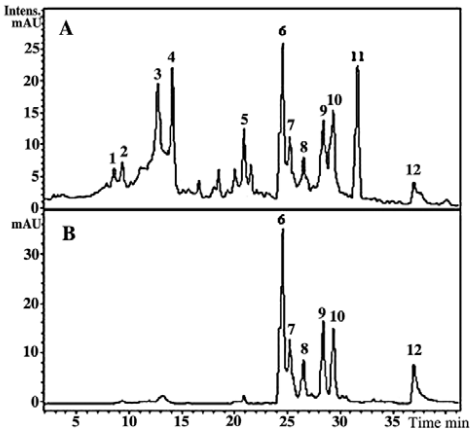



***Figure 1.*** Weather conditions in summer 2006 and 2007 in South Estonia

In the apple peel extract six major polyphenols (catechin, chlorogenic acid (3-*O*-caffeoylquinic), quercitrin (quercetin-3-rhamnoside), quercetin-3-galactoside, quercetin and phloridzin (phloretin-2-*O*-glucoside)) were identified by their chromatographic retention times and $MS^2$ fragmentation spectra when compared to the respective parameters of commercial standards. Standard chemicals for identifying and quantifying catechin, quercetin, quercetin-3-rhamnoside (quercitrin), quercetin-3-galactoside (hyperin), 3-*O*-caffeoylquinic acid (chlorogenic acid) and phloretin-2-*O*-glucoside (phloridzin) as well as formic acid were sourced from "Sigma-Aldrich Buchs" Co. (Switzerland). Methanol and HPLC grade were obtained from "Rathburn Chemicals" Ltd. (Walkerburn, UK). Six more polyphenols were putatively identified, but not quantified, by their fragmentation spectra. Sample UV-chromatograms at two wavelengths are presented in Figure 2. (-)-epicatechin was not quantified because of the same retention time with cyanidin-3-glucoside.

Peel (approximately 1 mm thick and weighing 0.2 g) at room temperature was cut from ten apples in three replications per cultivar, and rapidly transferred into a tube with 1 ml 0.01 M HCl in methanol. The samples were shaken for 30 minutes at 40 rpm using "Biosan Multi RS60". After shaking, the extract was transferred to another tube before second and third re-extractions of the peel using the same procedure as described above. The total extract (3 ml) was centrifuged by "Eppendorf Centrifuge 5810R" ("Eppendorf" AG, Germany) for 15 minutes at 4000 rpm. The extract was then cooled to 15ºC and 1 ml of extract was filtered through 0.45 µm "Millex-FH" filter and sealed into an airtight glass capsule ("Agilent", USA).

"Agilent 1100 Series HPLC" device equipped with a reversed phase Zorbax 300SB-C18 column (2.1 × 150 mm, 5 µm particle size – "Agilent", USA), photodiode array detector and an electrospray ionization ion trap MS/MS detector ("1100 Series LC/MSD Trap-XCT", "Agilent Technologies", Germany), operated in negative mode ionization (m/z interval 100–1000 amu, target mass – 400 amu) was used for identification and quantification of polyphenols from the filtered peel extract. The column was eluted at 0.3 ml per minute with gradient of 0.1% aqueous formic acid (solvent A) and of acetonitrile (solvent B) from 5 to 95%. The *HPLC 2D ChemStation* software with a *ChemStation Spectral SW* module ("Agilent Technologies", Germany) was used for the process guidance and primary processing of the results. The concentration of polyphenols in the apple peel was calculated according to respective calibration curves obtained using standard compounds and expressed as mg 100 $g^{-1}$ fresh weight (FW). The estimated content of total polyphenols was calculated as the areas under chromatographic curves (AUC) at $\lambda = 280$ nm.




*Note.* Peak identifications: 1) (+)-catechin ($[M-H]^- = 289$), 2) chlorogenic acid ($[M-H]^- = 353$), 3) procyanidin B2 ($[M-H]^- = 577$), 4) (-)-epicatechin ($[M-H]^- = 289$) and cyanidin-3-glucoside ($[M-H]^- = 447$), 5) feroylquinic acid ($[M-H]^- = 367$), 6) quercetin-3-galactoside ($[M-H]^- = 463$), 7) quercetin-3-glucoside ($[M-H]^- = 463$), 8) quercetin pentoside isomer 1 ($[M-H]^- = 433$), 9) quercetin pentoside isomer 2 ($[M-H]^- = 433$), 10) quercitrin ($[M-H]^- = 447$), 11) phloridzin ($[M-H]^- = 435$), 12) quercetin ($[M-H]^- = 301$).

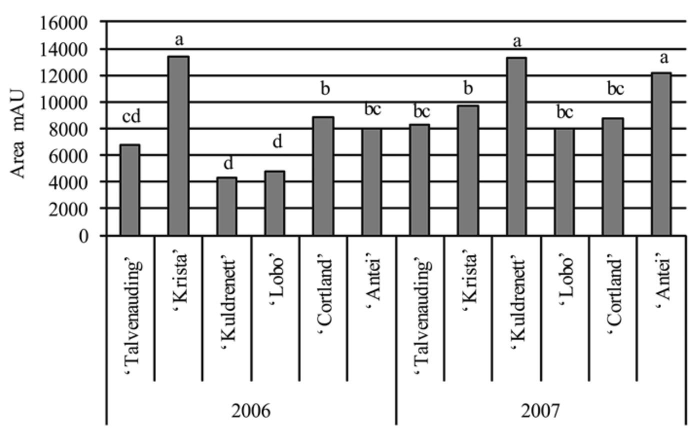
***Figure 2.*** UV-chromatograms of the peel extract of 'Antei' (2007) at 280 (A) and 370 (B) nm

*Statistical analyses.* One-way analysis of variance was used for testing the effect of the rootstock or cultivar on the polyphenols concentration. The means were separated by the least significant difference (LSD) test and differences at $P \leq 0.05$ were considered statistically significant.

## Results and discussion

***The effect of the genotype.*** The estimated content of total polyphenols varied significantly among the years and cultivars. In 2006, the total content of polyphenols was significantly higher in 'Krista', but in 2007 in 'Liivi Kuldrenett' and 'Antei' (Fig. 3). No significant differences were seen among other cultivars. The mean effect of the cultivar showed significantly higher content of total polyphenols in 'Krista'.

In the current study, phloridzin and quercitrin were the most abundant polyphenols in the apple peel. Polyphenols occurred in the following decreasing order: phloridzin > quercitrin > catechin > chlorogenic acid > quercetin galactoside > quer-




*Note.* Mean values followed by the same letter are not significantly different at $P \leq 0.05$.

***Figure 3.*** The estimated content of total polyphenols measured at $\lambda = 280$ nm in the apple peel of different cultivars grown in 2006 and 2007 in Estonia

cetin. Genotype had no significant effect on the order of polyphenols in the apple peel. These results are similar to those found by Lu and Foo (1997). D'Abrosca et al. (2007) also found that apple peel was rich in phloridzin, phloretin-2′-xyloglucoside, catechin and epicatechin.

The effect of genotype on the content of polyphenols was significant, but differed between the years (Table 1). In 2006, 'Krista' had significantly higher concentration of phenolic compounds, except for chlorogenic acid and phloridzin, compared to other cultivars. In 2007, 'Krista' had significantly lower concentrations catechin, quercetin and quercetin galactoside and no significant differences among other polyphenols compared to 2006.

***Table 1.*** The concentration of phenolic compounds (mg 100 g$^{-1}$ FW) in the peel of different apple cultivars grown in 2006 and 2007 in Estonia

| Cultivars | CAT | CHL | PHL | QERT | QGAL | QTRI |
|---|---|---|---|---|---|---|
| 2006 | | | | | | |
| 'Talvenauding' | 59.0g | 41.5de | 149.8d | 17.7c | 16.3c | 91.5d |
| 'Krista' | 123.9b | 52.5cd | 134.2de | 117.6a | 50.4a | 127.1c |
| 'Liivi Kuldrenett' | 45.3g | 27.9e | 108.0e | 5.2d | 1.1e | 26.9f |
| 'Lobo' | 52.2g | 25.0e | 120.0e | 5.6d | 1.7de | 36.0ef |
| 'Cortland' | 70.9f | 47.3d | 169.9cd | 35.0b | 17.5c | 77.6d |
| 'Antei' | 88.8de | 41.2de | 152.9cde | 17.5c | 12.5c | 64.2de |
| 2007 | | | | | | |
| 'Talvenauding' | 93.0de | 131.6a | 294.9a | 7.0d | 33.0b | 198.2b |
| 'Krista' | 98.0de | 69.0c | 141.1de | 11.1d | 37.1b | 101.5c |
| 'Liivi Kuldrenett' | 118.4bc | 123.7a | 293.4a | 22.5c | 16.8c | 270.0a |
| 'Lobo' | 81.3ef | 68.5c | 192.9c | 5.8d | 5.8de | 126.4c |
| 'Cortland' | 106.0cd | 104.6b | 279.1ab | 6.8d | 9.2d | 113.7c |
| 'Antei' | 157.9a | 160.2a | 249.5b | 8.5d | 39.5b | 203.4b |
| $LSD_{0.05}$ | 18.3 | 19.2 | 41.2 | 7.9 | 7.9 | 31.1 |

*Notes.* Mean values followed by the same letter are not significantly different at $P \leq 0.05$. CAT – catechin, CHL – chlorogenic acid, PHL – phloridzin, QERT – quercetin, QGAL – quercetin galactoside, QTRI – quercetrin.

In 2006, 'Antei' had significantly lower concentration of catechin and 'Liivi Kuldrenett' significantly lower content of quercetin. However in 2007, the content of those polyphenols was markedly higher compared to other cultivars and the year 2006.

The experiment demonstrated a large difference in the concentration of polyphenols in different apple cultivars showing the important influence of genotype. The largest variation of concentrations between the cultivars occurred in quercetin and quercetin galactoside. In 2006, 'Krista' and 'Lobo' had a 23-fold difference in quercetin concentration. Earlier experiments with 19 apple cultivars in Poland have shown a 9-fold difference in chlorogenic acid (Łata et al., 2009) and an experiment with three apple cultivars in Japan reported a 15-fold difference in catechin content (Kondo et al., 2002). Researchers have indicated the beneficial effect of quercetin on human health (Knekt et al., 2002; Ansari et al., 2009). The current study showed that the concentration of quercetin significantly differed among cultivars and years. In 2006, the concentration of quercetin and quercetrin was significantly higher in 'Krista' and in 2007, in 'Liivi Kuldrenett' (Table 1). The cultivar experiment showed that the apple peel with higher content of total polyphenols had also higher content of flavonols and catechin. Wojdyło et al. (2008) also demonstrated that genotypes with the highest phenolic concentration had simultaneously high content of flavonols.

Yearly different weather conditions had an impact also: a significantly higher concentration of phenolic compounds was found in 2007 (Fig. 3). We can assume that increased content of polyphenols in 2007 was caused by longer sunlight hours, less precipitation and higher temperature in August 2007 compared to the August 2006. Genetic background, developmental stage and environmental factors, such as nutrient availability, temperature and particularly light influence the synthesis of polyphenols (Saure, 1990). Sunlight induces many enzymes involved in flavonoid synthesis (Treutter, 2001). In 2007, all the apples had a better red skin colour and higher polyphenol concentration (except quercetin). Even the yellow apples of 'Liivi Kuldrenett' had a red blush, which might have been due the 68% more sun light hours in August 2007 compared to the same period in 2006. Better light conditions before harvesting probably caused higher polyphenols concentration in the apple peel in 2007. These results are in agreement with Hagen et al. (2007) who found that the red to green colour values of the apple skin correlated very well with the peel content of phenolic compounds and ascorbic acid in 'Aroma' apples. They also implied that the colour of the apple skin might provide useful information about the health value of the apples.

***The effect of the rootstock.*** The area of a peak indicating the estimated content of total polyphenols in the rootstock experiment ranged from 6781 to 15029 mAU min. No significant differences were seen between the years and vegetative rootstocks. The estimated content of total polyphenols was significantly lower only in 'Talvenauding' apples grafted on 'Antonovka' seedlings (data not shown).

The rootstocks significantly influenced the concentrations of different polyphenols in the apple peel (Table 2). Apples grown on vegetative rootstocks had significantly higher concentration of polyphenols compared to those grown on 'Antonovka' seedlings. In 2006, the concentrations of quercetin (82.5 mg 100 $g^{-1}$ FW) and quercetin galactoside (46.3 mg 100 $g^{-1}$ FW) in the apples grown on B.396 were significantly higher compared to the apples grown on M.26 where the concentration of same compounds were 71.7 mg 100 $g^{-1}$ FW and 30.9 mg 100 $g^{-1}$ FW, respectively. In 2007, the content of catechin, quercetin galactoside and quercitrin was higher in apples grown on B.396.

In both experimental years, significantly higher concentrations of phloridzin were detected in the peel of apples grown on vegetative rootstocks (Table 2). Also in both years there were no significant differences between vegetative rootstocks and 'Antonovka' seedling in chlorogenic acid concentration. Significantly higher concentration of quercetin galactoside was measured in apples grown on B.396 in both years. In 2006, more significant differences between vegetative rootstocks and 'Antonovka' seedling occurred. Significantly higher concentrations of catechin, phloridzin, quercetin and quercetin galactoside were measured in apples grown on vegetative rootstocks.

***Table 2.*** The concentration of phenolic compounds (mg 100 $g^{-1}$ FW) in the peel of 'Talvenauding' apples grown on different rootstocks in 2006 and 2007 in Estonia

| Rootstock | CAT | CHL | PHL | QERT | QGAL | QTRI |
|---|---|---|---|---|---|---|
| | | | 2006 | | | |
| Seedling | 59.0c | 41.5b | 149.8d | 17.7c | 16.3c | 91.5d |
| B396 | 91.0b | 50.8b | 217.4c | 82.5a | 46.3a | 116.8d |
| M26 | 111.5b | 58.8b | 195.1c | 71.7b | 30.9b | 128.1d |
| | | | 2007 | | | |
| Seedling | 93.0b | 131.6a | 294.9b | 7.0d | 33.0b | 198.2c |
| B396 | 143.1a | 139.8a | 370.0a | 16.1cd | 43.7a | 386.0a |
| M26 | 106.8b | 129.9a | 338.1a | 10.6cd | 28.8b | 289.1b |
| $LSD_{0.05}$ | 25.4 | 23.3 | 36.5 | 9.7 | 8.9 | 68.0 |

*Notes.* Mean values followed by the same letter are not significantly different at $P \leq 0.05$. CAT – catechin, CHL – chlorogenic acid, PHL – phloridzin, QERT – quercetin, QGAL – quercetin galactoside, QTRI – quercetrin.

Previous research conducted in Estonia demonstrated that dwarfing vegetative rootstocks successfully inhibit the growth of vigorous cultivars (Haak, 2003). Since 'Talvenauding' is a vigorous cultivar, the dwarfing effect of B.396 and M.26 might have had a beneficial influence on the concentrations of polyphenols, because canopies with weaker shoot growth provide better light conditions for fruits. According to Hagen et al. (2007) the concentration of flavonoids and the level of total phenols are higher in the peel of sun-exposed apples compared to shade-grown apples.

The rootstocks also significantly influence the intake of mineral elements from the soil. Studies have shown that the content of macronutrients in the leaves depend on the rootstock (Poniedziałek et al., 1993). Sotiropoulos (2008) found a higher concentration of N in the leaves of 'Imperial Double Red Delicious' grown on the seedling compared to the rootstocks M.7 and MM.106. Dris and Niskanen (1998) found a positive correlation between fruit and leaf N concentration and Awad and de Jager (2002) found a negative correlation between nitrogen and apple skin cyanidin-3-galactoside and total flavonoids concentration. Fallahi and Simons (1993) found that trees on M.7 had significantly lower leaf and fruit N contents which resulted in a darker fruit colour than that of fruits from trees on M.26 rootstocks, both at harvest and after storage. The intake of mineral elements from the soil is also significantly influenced by the soil moisture. It is also observed that rootstocks with different vigour have different ability to extract soil water (Lo Bianco et al., 2008). Less vigorous rootstocks have weaker root system and are therefore usually more susceptible to the weather conditions. However, in the current experiment it was observed that apples grown on vegetative rootstocks had higher content of most polyphenols compared to the seedling rootstock irrespective of the yearly different weather conditions.

## Conclusions

1. The current study confirmed that the genotype is a major factor influencing the concentration of polyphenols, since a 23-fold difference in polyphenol concentrations between the cultivars was observed. The obtained results may be used in the selection of apple genotypes suitable for Estonia and other countries with similar climate that have improved nutritional quality for fresh consumption. Among the studied cultivars, 'Krista' and 'Liivi Kuldrenett' had significantly higher concentrations of quercetin and quercitrin compared to all other cultivars.

2. The hypothesis about the influence of the rootstock on the apple polyphenols was confirmed: both vegetative rootstocks promoted formation of most polyphenols in 'Talvenauding' fruits. Among dwarfing rootstocks, B.396 was superior compared to M.26 in terms of increasing quercetin galactoside content in the peel of 'Talvenauding' apples. Further research is needed for other rootstocks and other cultivars. Yearly different weather conditions affected polyphenol content in apples, but fruits grown on vegetative rootstocks had higher content of most polyphenols compared to the seedling rootstock irrespective of the weather.

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# Genotipo ir poskiepių įtaka atrinktų obels veislių polifenolių sudėčiai Estijoje

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## Santrauka

Tyrimų, atliktų 2006–2007 m., tikslas – nustatyti pagrindinių polifenolių koncentraciją ir bendrą polifenolių kiekį Estijoje auginamų šešių obels (*Malus* x *domestica* Borkh.) veislių: 'Talvenauding', 'Krista', 'Liivi Kuldrenett', 'Lobo', 'Cortland' ir 'Antei', vaisių odelėje. Be to, tirta poskiepių B.396 bei M.26 ir veislės 'Antonovka' sėjinukų įtaka polifenolių kiekiui veislės 'Talvenauding' obuoliuose. Efektyvioji skysčių chromatografija (ESCH) buvo taikyta nustatant šių polifenolių kiekį: katechinų, chlorogeninės rūgšties, floridzinų, kvercetinų, kvercetinų galaktozido ir kvercitrinų. Polifenoliai buvo identifikuoti taikant tandeminę masių spektrometriją (MS/MS).
Polifenolių kiekį lėmė veislės genotipas, poskiepis ir metų oro sąlygos. Tarp veislių labiausiai skyrėsi kvercetino ir kvercetinų galaktozido koncentracija. Veislių 'Krista' ir 'Lobo' kvercetino koncentracija skyrėsi 23 kartus ir sudarė atitinkamai 117,6 ir 5,8 mg 100 $g^{-1}$ žalios masės. Obuolių odelėje polifenolių kiekis mažėjančiai pasiskirstė taip: floridizinai > kvercitrinai > katechinai > chlorogeninė rūgštis > kvercetinų galactozidas > kvercetinai; genotipas šiam pasiskirstymui neturėjo įtakos. Veislės, pasižyminčios didesniu kiekiu katechinų ir flavonolų, taip pat turėjo didesnį kiekį polifenolių. Polifenolių koncentracija veislės 'Talvenauding' obuoliuose buvo didesnė vaismedžių su vegetatyviniais poskiepiais, palyginti su vaismedžių su sėkliniais poskiepiais obuoliais.

Reikšminiai žodžiai: *Malus* x *domestica*, katechinai, chlorogeninė rūgštis, floridizinas, flavonolai, ESCH.

# IV

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THE EFFECT OF PRE-HARVEST CA TREATMENT ON CONCENTRATION OF POLYPHENOLS AND ANTIOXIDANT CAPACITY OF 'PIRJA' AND 'MAIKKI' APPLES GROWN ON DIFFERENT ROOTSTOCKS.
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Short communication

# The effect of preharvest Ca treatment on concentration of polyphenols and antioxidant capacity of 'Pirja' and 'Maikki' apples grown on different rootstocks

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ABSTRACT

The aim of the experiment was to determine the effect of preharvest $CaCl_2$ treatment on the concentration of certain polyphenols (catechin, chlorogenic acid, phloridzin, quercetin, quercetin galactoside, quercitrin and cyanidin glucoside) and on total antioxidant capacity (TAC) in 'Pirja' and 'Maikki' apples grown on rootstocks M.26 and B.396.

The results showed that the Ca treatment affected the concentration of polyphenols in apples from B.396 and M.26 differently; on B.396, the Ca treatment increased the concentration of quercitrin in apples of both cultivars, whereas on M.26, it decreased the concentration of quercitrin. Quercitrin, quercetin galactoside and quercetin were the major polyphenols contributing to the apple TAC. The concentration of these polyphenols was increased by higher fruit Ca and lower N concentration in 'Maikki'.



## 1. Introduction

Preharvest Ca treatment is agricultural practice to increase the concentration of Ca in apples (*Malus domestica* Borkh.) for better storability (Dris and Niskanen, 1998; Kadir, 2004; Saure, 2005; Moor et al., 2006). Preharvest Ca sprays also affect other quality parameters in apples. A positive effect on apple phenolics was observed by Sannomaru et al. (1998), who found that content of epicatechin, chlorogenic acid and total polyphenol of 'Starking' apples was higher in Ca treated than in untreated fruit. Awad and De Jager (2002) found an occasional positive correlation between Ca and total flavonoids in apple skin. They also stated that the most important variable in predictive models for the anthocyanin and total flavonoids concentration was N concentration in the fruit. The concentrations of cyanidin 3-galactoside (anthocyanin), catechins and total flavonoids were generally decreased by increasing the amount of N fertilizer (Awad and De Jager, 2002). It appears that among mineral elements, Ca and N play key-roles in apple quality.

Major polyphenols found in apples are quercetin glycosides, catechin, epicatechin, procyanidins, phloridzin, chlorogenic acid and anthocyanin (Lee et al., 2003; Hagen et al., 2006). The synthesis of these compounds is, in addition to genetic background, influenced by environmental factors such as nutrient availability, temperature, light (Saure, 1990; Treutter, 2001; Hagen et al., 2007), cultivation methods such as canopy pruning and fertilization, and rootstock (Awad and De Jager, 2002; Scalzo et al., 2005). Awad and De Jager (2002) found a negative correlation between nitrogen and apple skin cyanidin-3-galactoside and total flavonoids concentration. Earlier studies have shown that 'Talvenauding' apples grown on rootstocks B.396 and M.26, had significantly higher concentration of polyphenols compared to those grown on 'Antonovka' seedling (Mainla et al., 2011).

The total antioxidant capacity (TAC) varies among fruits of different species and is strongly influenced by genotype (species or cultivar). In apricots and peaches, the TAC was also influenced by the rootstock (Scalzo et al., 2005). In apples, a positive correlation between TAC and total phenolic content has been reported (Wolfe et al., 2003; Drogoudi et al., 2008). Although most of the phenolics are reported to have antioxidant activity, the estimated contribution of quercetin glycosides to apple TAC is the highest among major phytochemicals (Lee et al., 2003).

The current study was performed to test the hypothesis that, by using foliar Ca treatment, it is possible to affect apple polyphenol concentration and, with that, also the total antioxidant capacity. We also hypothesized that different rootstocks might interfere with the effect of Ca. The aim of the experiment was to determine the effect of preharvest Ca treatment on the concentration of certain polyphenols and TAC of 'Pirja' and 'Maikki' apples grown on rootstocks M.26 and B.396. Such information is important for developing cultural practices, which can improve the health related properties of apples.

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**Table 1**
The effect of the rootstock and Ca treatment on the concentration (mg 100 $g^{-1}$ FW) of polyphenols in the skin of 'Pirja' and 'Maikki' apples.

| Rootsock and treatment | CAT[a] | CHL | PHL | QERT | QGAL | QTRI | CYGL |
|---|---|---|---|---|---|---|---|
| 'Pirja' | | | | | | | |
| B.396 control | 91.1a | 184.0a | 113.9b | 3.7a | 22.1ab | 106.6b | 128.1a |
| B.396 Ca | 84.5a | 185.8a | 126.6b | 3.4a | 27.6a | 131.1a | 114.4a |
| M.26 control | 89.1a | 170.3a | 150.2a | 3.4a | 28.6a | 104.8b | 78.2b |
| M.26 Ca | 89.1a | 168.5a | 121.5b | 3.5a | 15.3b | 83.5c | 112.8a |
| $LSD_{0.05}$ | 14.0 | 19.3 | 21.8 | 0.4 | 7.9 | 12.7 | 21.0 |
| 'Maikki' | | | | | | | |
| B.396 control | 80.3c | 234.3c | 53.3c | 3.4a | 12.3ab | 243.4b | 99.0b |
| B.396 Ca | 109.3a | 317.3a | 88.4a | 3.9a | 11.9b | 290.9a | 119.2b |
| M.26 control | 102.0ab | 278.5b | 72.4b | 4.0a | 14.3a | 307.8a | 283.6a |
| M.26 Ca | 93.0b | 300.9a | 74.8b | 3.4a | 11.9b | 176.9c | 115.7b |
| $LSD_{0.05}$ | 12.5 | 29.6 | 13.6 | 0.9 | 2.4 | 19.9 | 36.9 |

Mean values followed by the same letter are not significantly different at $P \leq 0.05$.
[a] CAT, catechin; CHL, chlorogenic acid; PHL, phloridzin; QERT, quercetin; QGAL, quercetin galactoside; QTRI, quercitrin; CYGL, cyanidin glucoside.

## 2. Materials and methods

### 2.1. Plant material

The experiment was carried out in 2008 in South Estonia (58°21′N, 26°31′E) with Finnish apple cultivars Pirja and Maikki grafted on rootstocks M.26 and B.396. Trees were planted in 2001 with 2 m between the trees and 4 m between the rows in a randomized complete block design with four replicates and five trees per replication. The following foliar Ca treatments were used in 2008: (1) control (no calcium treatment); (2) $CaCl_2$ (0.5% $CaCl_2$ solution, 1000 L $ha^{-1}$) sprayed onto the trees on 22 June, 3 and 15 July. The original plan was to repeat the experiment in 2009, but since there was an enormous amount of snow in winter, the plantation suffered from flooding in the spring, which caused abnormal growth of leaves and shoots because of water stress. Several trees later died and had to be replaced. Under these circumstances the experiment could not be repeated.

### 2.2. Fruit harvest and preparation for analyses

'Pirja' apples were harvested on 21 July and 'Maikki' on 6 August. Harvest maturity was determined by seed color (the tips of the seeds turned brown) and confirmed by the iodine-starch test (a narrow area of the fruit flesh (2–3 mm) under the skin turned blue). Apples were picked according to an equatorial pattern (North–South–East–West) from the outside of the trees avoiding fruit situated at the top, the bottom and also deep inside the canopy. The yield was harvested from all experimental trees. From the total yield three samples (3 × 5 kg fruits) was randomly taken for analyses. For determination of polyphenols, mineral elements and TAC, 10 fruits per replicate were selected randomly for each analysis. Before the analyses, apples were cooled for 24 h in a cool store at 6 °C.

### 2.3. Determination of mineral elements, polyphenols and TAC

For Ca and N analysis apples were cut into sectors, seeds and stems were excluded but skin was included in the analysis. Apple N concentration was determined by the Kjeldahl method (Benton, 2001) and Ca concentration by an induction couplet plasma spectrometer. Nutrient concentrations were expressed as mg $kg^{-1}$ fresh weight (FW).

In the apple skin extract seven major polyphenols, catechin (CAT), chlorogenic acid (CHL), quercitrin (QTRI), quercetin galactoside (QGAL), quercetin (QERT), phloridzin (PHL) and cyanidin glucoside (CYGL), were determined as previously described by Mainla et al. (2011).

Ethanolic apple extract was used for TAC determination by the 1.1-diphenyl-2-picrylhydrazyl (DPPH) discoloration assay described by Brand-Williams et al. (1995) with some modifications. An aliquot of apple extract at different concentrations was added to the DPPH solution and the absorbance at 515 nm was measured during 120 min (until the reaction had reached a plateau). The $EC_{50}$ parameter, the amount of sample necessary to decrease by 50% the initial DPPH concentration, was calculated for the apple extracts. For the calibration curve ascorbic acid solutions at different concentrations were used. The results of TAC were expressed as mg ascorbic acid equivalent (AAE) 100 $g^{-1}$ FW as suggested by Kim et al. (2002).

### 2.4. Statistical analyses

To determine the effect of the Ca treatment on different rootstocks, we used one-way analysis of variance. The means were separated by the least significant difference (LSD) test and differences at $P \leq 0.05$ were considered statistically significant. To study the relationship between fruit N and Ca content and concentration of polyphenols, correlation analysis was used. Regression analysis

**Table 2**
The correlation coefficient ($r$) between concentrations of different polyphenols (mg 100 $g^{-1}$ FW), total antioxidant capacity (mg AAE 100 $g^{-1}$ FW), N and Ca concentration (mg $kg^{-1}$ FW) and N/Ca ratio in 'Maikki' apples.

| 'Maikki' | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mineral content and ratio | CAT[b] | CHL | PHL | QERT | QGAL | QTRI | CYGL | TAC |
| Ca | ns[a] | ns | 0.697* | 0.731* | 0.768* | 0.792* | ns | 0.804** |
| N | −0.909*** | −0.804** | −0.990*** | −0.847** | ns | ns | ns | ns |
| N/Ca | −0.693* | ns | −0.930*** | −0.871** | ns | −0.666* | ns | −0.740* |

[a] Not significant.
[b] CAT, catechin; CHL, chlorogenic acid; PHL, phloridzin; QERT, quercetin; QGAL, quercetin galactoside; QTRI, quercitrin; CYGL, cyanidin glucoside; TAC, total antioxidant capacity.
* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$, $n = 12$.

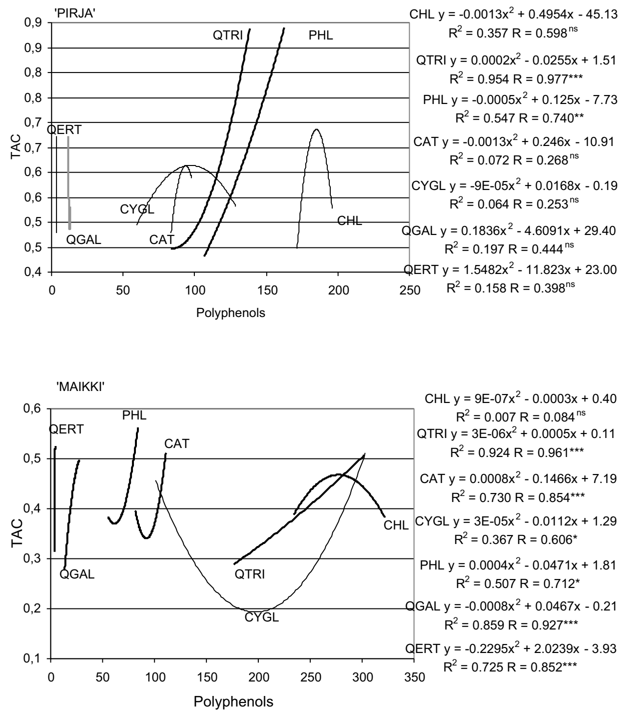


**Fig. 1.** The effect of polyphenols (mg 100 g$^{-1}$ FW): catechin (CAT), chlorogenic acid (CHL), phloridzin (PHL), quercetin (QERT), quercetin galactoside (QGAL), quercitrin (QTRI) and cyanidin glucoside (CYGL) on the total antioxidant capacity (TAC) mg AAE 100 g$^{-1}$ FW in 'Pirja' and 'Maikki' apples. *Significant at $P \leq 0.05$, **significant at $P \leq 0.01$, ***significant at $P \leq 0.001$, ns = not significant, and $n = 12$.

was used to determine the relationship between the fruit polyphenols and total antioxidant capacity. The coefficient of determination ($R^2$) was calculated.

## 3. Results and discussion

On B.396, the Ca treatment significantly increased the concentration of QTRI in both cultivars (Table 1); in 'Pirja', the increase was 23% and in 'Maikki' 20%. In 'Maikki' on B.396, the Ca treatment also increased the concentration of CAT, CHL and PHL. On M.26, the Ca treatment significantly decreased the concentration of QTRI in both cultivars, causing a 43% decrease in 'Maikki' and 20% in 'Pirja'. In the latter cultivar on M.26, the Ca treatment also significantly decreased the concentration of PHL and QGAL and increased only the concentration of CYGL. In 'Maikki' on M.26, the Ca treatment also significantly decreased QGAL and CYGL concentrations.

Thus, the research results confirmed the hypothesis that Ca treatment has a significant effect on apple skin polyphenol concentration, but the influence depends on the rootstock. In fruits grown on B.396, several polyphenols were increased by the Ca treatment, whereas on M.26, the Ca treatment mostly decreased the concentration of polyphenols. The foliar Ca treatment was a beneficial tool to increase the polyphenol concentration in 'Maikki' on B.396. Similarly, our previous research indicated that winter cultivar 'Talvenauding' apples grown on rootstocks B.396 and M.26 had a significantly higher concentration of polyphenols in the skin compared to those grown on 'Antonovka' seedling (Mainla et al., 2011).

The correlation analysis indicated that in 'Maikki', Ca had positive significant correlations with QERT, QGAL, QTRI and PHL (Table 2). N showed significant negative correlation with QERT, CAT, CHL and PHL. N/Ca ratio also had significant negative correlations with QERT, QTRI, CAT and PHL. In 'Maikki', a significant positive correlation between TAC and Ca was found. TAC had a negative correlation with N/Ca ratio. In 'Pirja', no significant correlations were found with most of the polyphenols. Also, TAC had no correlation with the mineral concentration in 'Pirja' (data not shown).

In 'Maikki', significant nonlinear correlations between TAC and the majority of the polyphenols were found: PHL, QERT, QGAL, QTRI, CYGL and CAT (Fig. 1). Only CHL did not have significant correlation with TAC. In 'Pirja', only QTRI and PHL showed significant nonlinear correlations with TAC. The trendline showed an increase of TAC with increasing concentration of QTRI, but with increasing PHL concentration in apples the increase of TAC was slower.

Our findings clearly indicate that polyphenols have different antioxidant capacity, since the quantity of QERT in 'Maikki' apples was negligible compared to several other polyphenols, but even in such small amounts it had an effect on the apple TAC. Our results are also in agreement with previously reported findings from Hagen et al. (2007), who found, in 'Aroma' apples, the strongest correlation between TAC and quercetin glycosides (quercetin-3-galactoside, quercetin-3-glucoside and quercetin-3-rhamnoside).

Lee et al. (2003) showed with six apple cultivars (Golden Delicious, Cortland, Monroe, Rhode Island Greening, Empire, and NY674) that the estimated contribution of quercetin glycosides to the TAC is the highest among major phytochemicals, whereas chlorogenic acid and phloretin contribute minimally. In our experiment, PHL also provided a smaller though still significant contribution to TAC.

Although in the current experiment, the correlation analysis also showed a positive correlation between Ca and TAC in 'Maikki', it can be assumed that the influence of mineral elements on TAC is still through their influence on polyphenols. The relationship between TAC and Ca became evident in the correlation analysis because the largest contributors to TAC (QERT, QGAL and QTRI) also had a positive correlation with Ca. Also, no negative relationship between TAC and N was found for the reason that QGAL and QTRI had no correlation with N. Hence, the effect of cultural practices on apple TAC is through the relationships between mineral concentration and polyphenols and polyphenols and TAC.

## 4. Conclusion

In the current study, the more vigorous cultivar 'Maikki' was more responsive to foliar Ca treatment than 'Pirja'. The preharvest Ca treatment affected the concentration of polyphenols. Since polyphenols differ in their contribution to antioxidant capacity, it is also important to know, which compounds to affect. In the current study, QTRI was the major polyphenol contributing to apple TAC. On B.396, the Ca treatment significantly increased the concentration of QTRI in apples from both cultivars, whereas on M.26, it significantly decreased the concentration of QTRI.

Further research with different cultivars and rootstocks is necessary to provide the knowledge base enabling choice of agricultural practices that affect beneficial health properties in apples.

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# LIST OF PUBLICATIONS

### 1.1. Articles indexed by Thomson Reuters Web of Science

**Mainla, L.**, Moor, U., Karp, K., Tõnutare, T. 2012. The effect of preharvest Ca treatment on concentration of polyphenols and antioxidant capacity of 'Pirja' and 'Maikki' apples grown on different rootstocks. *Scientia Horticulturae* 148: 93–96.

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Moor, U., Karp, K., Põldma, P., **Asafova, L.**, Starast, M. 2006. Post-harvest disorders and mineral composition of apple fruits as affected by pre-harvest calcium treatments. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science* 56 (3): 179–185.

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### 3.5. Articles/presentations published in local conference proceedings

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## 6.3. Popular science articles

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Professor **Jüri Olt**

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